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DUTCH ELM DISEASE and its Chemotherapy

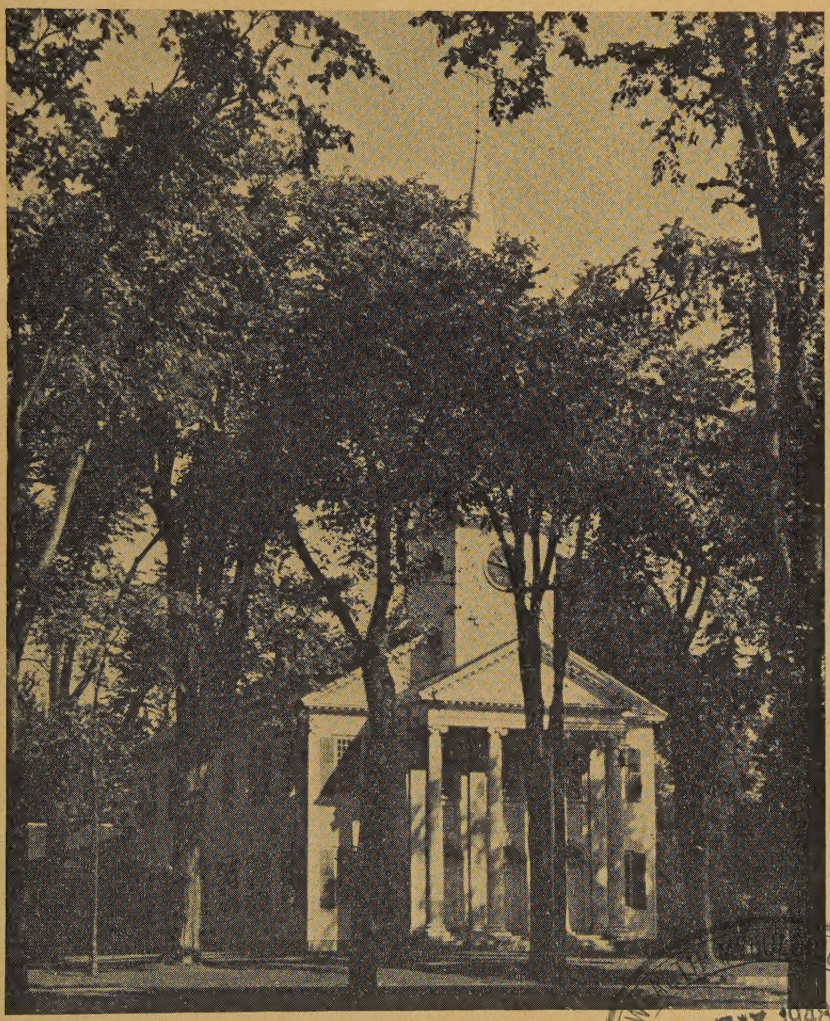

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Figure 1. Tree in late stages of Dutch elm disease.

DUTCH ELM DISEASE AND ITS CHEMOTHERAPY[1]

GEORGE A. ZENTMYER,[2] JAMES G. HORSFALL, PHILIP P. WALLACE[3]

The invasion of the Dutch elm disease into the United States in 1930 was a potential threat to thousands of valuable shade and ornamental elms throughout the nation. Vivid memories of the destruction wrought by the chestnut blight aroused fear that this new disease would similarly wipe out the elms. Since work on the disease in Europe had not developed any promising method of control, the obvious initial control effort here was aimed at total eradication. This eradication project grew in scope until 1938, but then declined and stopped almost entirely in 1940 after the onset of the world war.

Total eradication has been found impossible, and the emphasis on control of the disease has shifted to application of suppressive measures in specific local areas. The general tendency at present is to learn to "live with the disease". Unfortunately, little field research toward that end was possible during the 'thirties because such research requires the maintenance of diseased elms and this was clearly incompatible with the theory of total eradication.

In 1940 the Connecticut Agricultural Experiment Station expanded its research on various phases of the problem, with the object of discovering how best to "live with the disease". Considerable emphasis has been placed on control of the disease by chemical immunization or chemotherapy, a promising approach. Studies have been made also of the biology of the fungus, factors of inoculation and progress of the disease in the tree, factors in the long and short range spread of the disease, and of therapy by pruning.

In retrospect it is clear that the Dutch elm disease has not overrun the elms of the country during its first fifteen years nearly as rapidly as the chestnut blight killed the chestnut trees in its first fifteen years. One of the major objectives of this research has been to throw light on the reasons for this. Such light should illuminate the path toward improving control practices.

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METHODS

The study of the Dutch elm disease in nature is difficult because trees are scattered and because of the great variation between individual specimens. They differ widely in age, in type of development, and in nutrition. Therefore, much of the work reported herein was conducted with relatively small (between four and twelve feet tall) American elm trees (*Ulmus americana*) growing in nursery rows. The trees were about four feet tall when transplanted in 1941. Considerable field work, using swamp and roadside trees, was done on the local spread of the fungus and on chemotherapy and pruning.

Since elm trees are not as plentiful nor as easily produced as herbaceous plants, the size of the experimental sample caused some concern. In general, a 10-tree sample was used, sometimes randomized and sometimes used in two-tree lots. Presumably the error would have been reduced by using only random trees.

The recording of infection data is also of importance. The simplest statistic is the percentage of trees infected in any treatment, but usually inoculation was so drastic that all trees showed symptoms and the statistic became meaningless. The percentage of leaves showing disease was also estimated for each tree and an arithmetic average was obtained.

This statistic was found to be of considerable value for distinguishing between treatments. What it really does, of course, is to measure the distance to which the spores or the toxin of the fungus or both are able to travel within the tree. It must be remembered in appraising the data that the mean percentage of leaves diseased is not so much a measure of the probability of infection as of the probability that the fungus or its pathogenic toxin will reach the foliage. In other words, percentage of diseased leaves on a plant affected with a vascular fungus is not equivalent to percentage of diseased leaves affected with an air-borne fungus.

The percentage of die-back was also estimated, but the results probably are not different from those on percentage of affected leaves.

The terms "inoculate" and "inject" will be used frequently. To avoid confusion, they are defined in the sense in which they will be used here. To inoculate a tree is to introduce fungus spores or mycelium into it, generally through a wound. The purpose of inoculation is to produce disease. To inject a tree is to introduce a chemical into it, usually through a wound. The aim of injection is to immunize or to cure.

THE PRODUCTION AND TRANSPORT OF INOCULUM

The Dutch elm disease is caused by the fungus *Ceratostomella ulmi* (Schwartz) Buisman.

Nutrition of the Causal Fungus

In 1940, addition of growth substances was found to be essential for good mat formation by *C. ulmi* in synthetic liquid media. Sparse growth on Richard's, Leonian's, and Czapek's solutions was initially increased by addition of technical grade maltose, or orange juice, or liver extract (Extralin) containing vitamin B complex. Later, addition of brewers' yeast was found to promote very satisfactory growth; the thiamin chloride (vitamin B_1) fraction of the yeast was not an important factor, as single additions of this vitamin did not stimulate growth. "Bacto" yeast extract was subsequently used in place of brewers' yeast, and found to promote excellent growth. Work elsewhere (6, 25) has shown that pyridoxine (vitamin B_6) is the most essential of the yeast growth factors for *C. ulmi*.

Various nutrient combinations with yeast extract were tested to find the optimum liquid medium for the fungus. Either maltose or glucose are good carbon sources. Asparagine or peptone are more satisfactory nitrogen sources than ammonium nitrate, glycine, or potassium nitrate.

The following nutrient solutions are satisfactory for growth of the fungus: (1) KH_2PO_4 1. 5 g., $MgSO_4$.$7H_2O$ 1.0 g., glucose 25 g., asparagine 2 g., yeast extract 2 g., and distilled water 1,000 ml.; (2) same as No. 1, with 2 g. of peptone instead of asparagine; (3) Tochinai nutrient: KH_2PO_4 1.5 g., $MgSO_4$.$7H_2O$ 0.5 g., peptone 10 g., maltose 20 g., and distilled water 1,000 ml.

Excellent mat formation is obtained with decoctions of elm twigs or leaves, the former being made by cutting wood into small chips, boiling for 30 minutes, then either filtering off the chips or leaving them covered with the aqueous extract, and autoclaving. Extracts of both American elm and of Siberian elm (*U. pumila*) support growth, but growth is not as satisfactory on extracts of Siberian elm as on extracts of American. An excellent solid medium is malt extract agar, made with 20 g. of agar, 100 g. of ground malt barley, and 1,000 ml. of water. On malt agar the fungus produces masses of small yeast-like spores. These spores can be suspended easily in water to produce the inoculum for experimental work. Growth is good on potato dextrose agar and on oat agar.

C. ulmi requires various trace elements. Tochinai nutrient solution was purified of metals by precipitation with calcium carbonate and filtration while hot (27). Growth in the resulting solution was very poor. Growth was nearly normal in a solution containing the following trace elements at these proportions per liter of nutrient: .075 mg. of copper, .075 mg. of manganese, .30 mg. of iron, and .30 mg. of zinc. Omission of zinc alone resulted in much poorer growth; omission of iron alone had nearly as deleterious an effect. Omitting manganese had no effect, but omission of copper alone caused considerable increase in growth over the "complete" nutrient solution, indicating sensitivity of the fungus to copper.

Growth of the fungus under alternating conditions of light and dark (day and night) has been commonly observed to assume a zonate appearance on agar media. This phenomenon is apparently related to variable growth rate and variable character of growth in light and dark, as it does not appear when cultures are grown constantly in light or constantly in the dark, with varying temperatures (Figure 2). Growth is much faster in the dark than under fluorescent lights; the ultra-violet content of the light reaching the cultures after passing through the glass of the light tubes and of the petri dish covers should be insufficient to retard growth.

The fungus develops most rapidly in nutrient solutions within the range of pH 4 to pH 5. The following are mat weights after 10

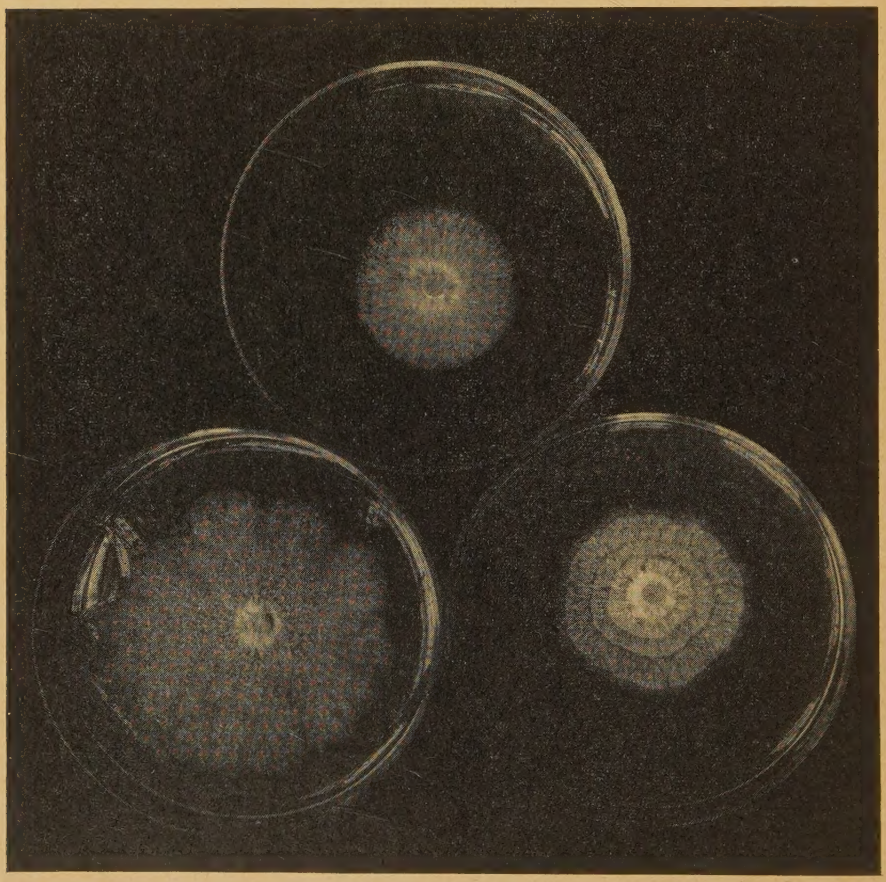

Figure 2. Effect of light on growth of *Ceratostomella ulmi* in culture. Culture at left was grown in complete darkness, culture at right was grown in alternating periods of light and dark, culture above was grown in continuous light. All three were subjected to variable room temperature. All three cultures were the same age when photographed.

days growth on Tochinai nutrient solution initially adjusted to the pH values given:

pH 2.8	3.2	3.8	4.5	5.9
28 mg.	43 mg.	156 mg.	208 mg.	120 mg.

Starting with a nutrient solution with a pH value of 5, growth of *C. ulmi* causes an initial drop in the pH of the nutrient solution to about pH 4 in two weeks. There is little variation in the hydrogen ion concentration for the next four or five weeks; then the acidity of the medium gradually decreases. At twelve weeks it was found to be still approximately 4.2; only after several months does the solution become alkaline. These results are similar to those found in investigations with other fungi.

Role of Beetles in the Transport of Inoculum

In nature *Ceratostomella ulmi* produces spores in the tunnels made in elm bark by bark beetles, chiefly *Scolytus multistriatus* Marsh. and *Hylurgopinus rufipes* Eichh. (23). It does not, so far as is known, produce spores on the outside of bark but does produce spores in the vessels in the wood. The spore masses produced in the larval tunnels are gelatinous. They become attached to the emerging adult beetles and as the beetles feed in nearby elms, the xylem vessels are severed and the spores are introduced into them. The fungus may be transmitted also through root grafts.

FACTORS INFLUENCING THE INITIATION OF DISEASE IN INDIVIDUAL ELMS

Method of Inoculation

In order to facilitate research on all phases of the disease problem, it was necessary to develop a simple and efficient method of artificial inoculation. This proved to be a relatively easy problem with the Dutch elm disease. The method used extensively here is a modification of that developed at the U. S. Department of Agriculture Dutch elm disease laboratory at Morristown, N. J. It is very satisfactory for inoculating small trees and upper branches of larger trees.

In all comparative experiments the inoculum used was taken from the same isolate of the fungus. Usually all inoculations in one season were made from successive mass transfers from the advancing margin of a single isolate made at the beginning of the season. In the conidial stage the fungus was found to be remarkably constant; sectoring was very uncommon on malt agar cultures. Walter (37) has shown, however, that variants of *C. ulmi* occasionally appear. Tyler and Parker (32) have recently noted considerable differences in pathogenicity between several isolates of *C. ulmi*, however, in their work in New York.

Introduction of a heavy suspension of fungus spores directly into cut xylem vessels is the most convenient type of artificial inoculation. Where possible the branch or top of the tree to be inoculated is pulled

down to a nearly horizontal position; a drop of spore suspension is placed on the upper surface of the branch with a medicine dropper or pipette, and a cut is made through the drop into the xylem with a sharp scalpel. The spore drop passes immediately in through the cut vessels; entrance is particularly rapid when large spring vessels are severed. Under optimum conditions, symptoms of the disease will appear within a week or 10 days, following the inoculation of a 10-foot tree with a heavy spore dose (10,000 spores or more) by the above method. This method can also be used for basal inoculations of trees which have not yet formed a thick corky bark and for inoculating the upper branches of large trees; the scalpel blade is placed horizontally against the tree trunk or branch, the spore drop is placed on the upper surface of the scalpel blade, and the cut is then made into the xylem. Banfield (1, 2) has demonstrated extremely rapid movement of *C. ulmi* spores through the large spring vessels during the growing period. Spores were recovered one hour after injection up to 62 feet in a 65-foot tree.

The common type of wound inoculation has also been used successfully with Dutch elm disease, but it is much more troublesome than the spore suspension method and does not usually produce as rapid disease development as the latter. Banfield (1) has reported similar results. In the wound type, a small piece of fungus mat with attached agar, from a petri dish culture, is placed in a vertical cut on the stem, the site of the inoculation is wrapped with wet cotton and kept moist for about 24 hours.

To study the relative effectiveness of spore and mycelial inoculations, 26 elms were inoculated on August 25, 1941, with spores, and 20 trees with mycelium. Twenty-six, or 100 per cent, of the trees receiving spores developed symptoms, the average wilt per tree by October 1 was 60 per cent, and the average die-back was 10 per cent. Only nine, or 45 per cent, of the trees inoculated with mycelium showed wilt, the average wilt per tree was 12 per cent, and the average die-back, 5 per cent.

Location of Inoculation Point

Artificial inoculations at the base of a tree were found to be more effective than those in the top or in side branches, presumably because the upward stream through the vessels is very efficient in spreading the spores and fungus toxin throughout the tree. Similar differences can be demonstrated between branches of different years' growth; inoculations in twigs of current season's wood usually develop to a lesser extent than those in older wood. Table 1 illustrates these points.

Banfield (3) has shown similar results; inoculations in the bole resulted in extensive vascular invasion, which is apparently correlated with his finding that the vessels in the bole are often many meters in length. He reported that vessels in new shoots were only a few centimeters long, and that little or no invasion occurred from inoculating

such shoots. Banfield also has reported (2) that spores moved downward in a tree more extensively in late summer or during the dormant season than in the main growing period; he did not present the effect of spore movement as measured by disease development.

Comparable results would be expected from natural inoculations; occasional inoculations in an elm trunk, by bark beetles, undoubtedly result in more severe disease conditions than do the more common inoculations in twig crotches.

TABLE 1. INFLUENCE OF LOCATION OF INOCULATION ON SUBSEQUENT DEVELOPMENT OF THE DUTCH ELM DISEASE

Date of inoculation	Location of inoculation	No. of trees inoculated	No. of trees wilting 1943	Average wilt by October 1, 1943 (per cent)	No. of trees wilting 1944	Average die-back by September 1944 (per cent)
July 2, 1943	Base	10	10	75	..	..
" " "	Top	10	10	20	..	..
July 31, 1943	Base	10	7	18	..	..
" " "	Top	10	4	3	..	..
July 10, 1943	Top-1943 wood	5	2	2	0	..
" " "	Top-1942 wood	5	4	27	4	25
" " "	Top-1941 wood	5	5	35	2	70

Note: Trees were 6-10 feet tall.

Time of Inoculation

Inoculation of more than 2,000 American elms at various times during the growing season, over the past four years, has provided information on the time of year at which trees are most susceptible to the disease, and on the relation of weather to development of infection. Most of the experiments were done on elms 5 to 12 feet tall, growing in a nursery block; larger trees up to 30 feet tall were used in other inoculations.

An extensive series in 1943 provided the most complete data. In this experiment 10 trees were inoculated about one foot above the base every week from May 1 to September 1. The scalpel method of inoculation was used; each tree was inoculated with approximately one million spores. Four times during the season, ten trees were also inoculated in the top by the same method.

The trees were examined individually in September and the percentage of diseased leaves was estimated. The data are shown graphically in Figure 3. The severity of infection increased to an optimum for early June inoculation and then decreased rapidly.

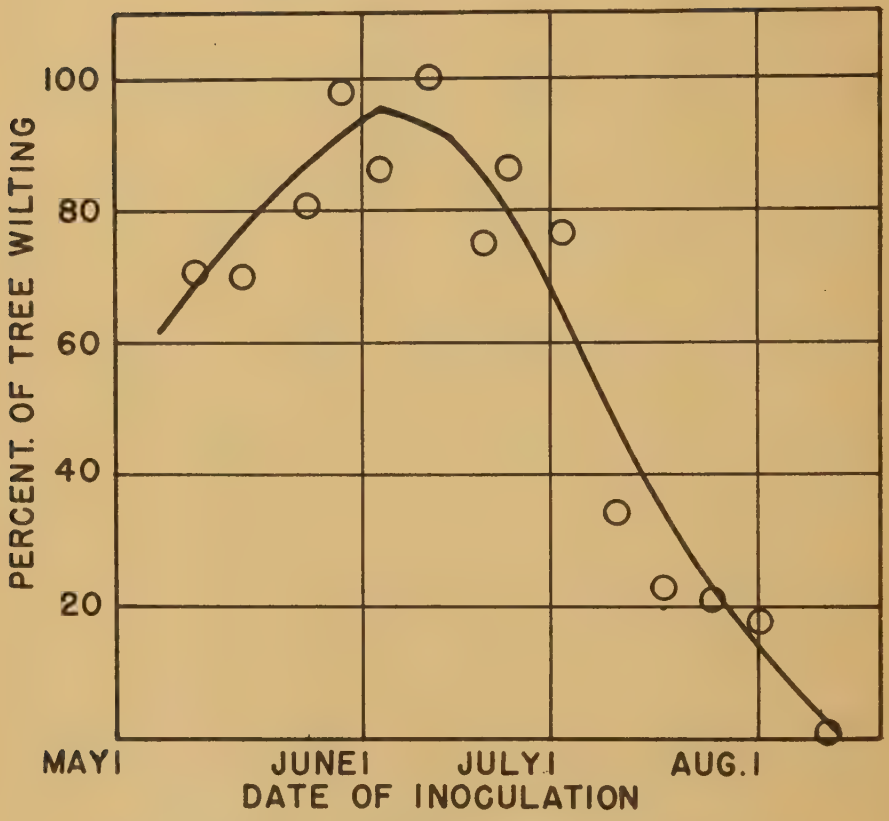

Figure 3. Effect of date of inoculation on development of Dutch elm disease.

At the time of the May 1 inoculations the leaf buds were expanding but had not opened. Only three out of 10 trees showed disease symptoms in this inoculation group, but these three averaged 95 per cent die-back by the end of the season. By May 7, leaves were about one-half inch long; only three of 10 trees became diseased, two heavily, one lightly. By May 14, leaves were one inch long and in some trees one or two rows of large spring vessels had been formed. Symptoms appeared in all of these trees. Development of the disease was not quite as severe as from inoculations made two weeks later. From May 14 through July 2, with the exception of June 12 (failure here may have been a matter of technique) inoculations were 100 per cent effective. A wide zone of spring vessels had been formed in some trees by June 4, with very little summer wood formation. There was an indication at this time that trees with wider zones of large spring vessels became more severely diseased. By June 25, several rows of smaller summer pores had been formed, and by July 17, summer wood made up the greater proportion of the annual ring. Extent of disease development reached a peak with the May 29 and June 5 in-

oculations, and then declined through June and fell sharply in July. None of the August inoculations developed external symptoms, although light streaking of the wood was common. These trees did not show symptoms in 1944, either.

As the result of observations made on the above series of trees and on various other groups of trees inoculated at varying times, the following generalizations can be made. Inoculations made during the active period of spring growth, after leaves have partly expanded, (generally from mid-May to late June) are the most effective, with respect to percentage of trees showing disease symptoms, and also to development of the disease. Before or after this period, the percentage of trees developing external symptoms is usually less. Development of the disease may be as severe in the case of *successful* inoculations made *before* this time, but is generally not as severe in the case of inoculations made after spring growth ceases (generally after the first week in July). Some variation, of course, may be expected with the season. (See below)

The weather in the year of inoculation has an influence on the percentage of trees developing symptoms and on the percentage of crown wilted per tree. In the summer of 1941 the precipitation was considerably above average; basal inoculations in July of this year resulted in 100 per cent infection, approximately 85 per cent of the crown wilted per tree and 70 per cent die-back by the end of the season. Continued radial growth of the trees under moist conditions may have contributed to the success of these summer inoculations. Inoculations in August, 1941, resulted in appearance of symptoms in over 50 per cent of the trees, an average percentage of crown wilted of 30 to 40 per cent, but only 5 per cent die-back per tree. In dry years, such as 1943 and 1944 were in southern Connecticut, growth is completed early and the effectiveness of inoculations falls off rapidly from early July. August inoculations in such years produce no external symptoms, neither in the year of inoculation nor in the following year. Even in years of high summer precipitation, inoculations in August invariably result in very slight die-back, although the percentage wilt may be appreciable.

It might be assumed that May inoculations produce more severe infections than August inoculations because the May inoculations have the advantage of a longer portion of the growing season in which to develop. This does not seem to be the case, however. The major progress of the disease in small trees takes place in the first month after inoculation. The greater development of the disease following early season inoculations is apparently correlated to some extent with the high proportion of large spring vessels in the current annual ring at that time. As Banfield (1) has shown, *C. ulmi* spores are transported readily through the tracheal tubes, and the transport both of spores and of fungus toxin should be more rapid and extensive through the large spring vessels than through the small summer vessels.

In natural inoculations it would seem that spores are less likely to be introduced into spring vessels by feeding of beetles in the summer, as the spring vessels are then buried somewhat by the thick layer of small-pored "summer" wood. Even if spores are inoculated directly into large spring vessels in the summer, they undoubtedly contact a sap stream of different composition than that encountered in spring.

The chemical composition of the spring wood is apparently an important factor. Hot water extracts of terminal growth, consisting entirely of 1944 spring wood, supported much better growth of the fungus in the laboratory than did extracts of two-year old twigs from which the 1944 spring wood had been peeled. The average dry weight of the fungus mat from cultures on 20 ml. of the extracts, after 15 days growth, was 11.6 mg. for the 1944 spring wood extract, and 5.1 mg. for the extract minus the 1944 spring wood.

These results indicate that early season emergence of *Scolytus* beetles (23) which disseminate the fungus, provides the most serious threat to elms, as the inoculations that they may cause at that time are much more likely to develop into serious infections than are inoculations made by the second brood which emerges in the summer. The results of experimental inoculations are supported also by observations in the field.

Effect of Number of Spores

Inoculum potential is an aspect of a disease that lends itself to quantitative appraisal. Heald (13) has shown a quantitative relation between number of spores per seed and percentage of infection of that seed by wheat bunt. If his data are plotted on logarithmic-probability paper (9), it is apparent that the logarithm of the number of spores bears a linear relation to the probability of infection.

It was of interest, therefore, to measure the effect of spore load on development of the Dutch elm disease. Following exploratory tests in 1943, a rather complete experiment was made in 1944 beginning in the early season on May 22. Ten elms from six to ten feet tall were inoculated in the top with spores produced in culture, using each of the following doses per tree: 10, 10^2, 10^3, 10^4, 10^5 and 10^6. The number of spores was checked with a haemocytometer. The average percentage of leaves wilting per tree was estimated on several occasions during the season. Data are given in Table 2. The year 1944 was not quite ideal for an experiment on a wilt disease because the rainfall for the season was the smallest during 70 years of records. Nevertheless, the data are interesting.

Even as few as 100 spores were sufficient to produce some disease in all 10 inoculated trees. If, however, only 10 spores were used, not every tree became visibly infected.

TABLE 2. EFFECT OF SPORE LOAD ON INFECTION BY DUTCH ELM DISEASE INOCULATED MAY 22, 1944

No. spores per tree	Average percentage of leaves wilted per tree[2]			
	June 12 21 days after inoculation	June 22 31 days after inoculation	July 31 70 days after inoculation	August 29 99 days after inoculation
10	5	13	48	48
100	10	17	60	70
1,000	12	37	70	74
10,000	38	62	66	73
100,000	39	57	77	85
1,000,000	45	56	72	84
10×10^1	14	25	38	45
100×10^1	75	75	70	78

[1] 10 inoculation points per tree.

[2] All trees showed some wilt except for the 10-spore lot, where only four became infected.

The proportion of diseased leaves in the infected trees increased with the spore load used for inoculation. The quantitative relation apparently is not a simple one, however. This relation will be discussed below in the section on "Progress of the disease in an individual sick tree".

Effect of Number of Inoculation Points

From these studies on spore load, it follows that a beetle must carry about 100 spores if he is to enjoy high odds of successfully inoculating a tree six to ten feet high. Nothing is known yet on the odds for inoculating big trees. Perhaps a single beetle may not carry sufficient inoculum but, on the other hand, it is probable that in nature a large number of beetles carrying a few spores each may be available to inoculate any given tree. Therefore, an experiment was designed to differentiate the effects of inoculating any given number of spores at one point or divided among several points of inoculation.[1]

Two tests were made in 1944. One test of single versus multiple inoculation was included in the May 22 trials on spore load mentioned in the previous section (Table 2). The 10^2 load was compared at one inoculation point of 100 spores with 10 inoculation points of 10 spores each. The 10^4 load was compared at one inoculation point and at 10 inoculation points of 1,000 spores each. All trees became infected in both comparisons, but the multiple inoculation was more successful in advancing through the tree than the single inoculation.

[1] Some theoretical aspects of the experimental design had already been worked out with fungicides (14, 30). The farmer wishes to know whether it is more efficient to use a relatively few gallons of concentrated spray or many gallons of relatively weak spray. Here, the question is whether a tree is more liable to infection from a concentrated dose of spores inoculated at one site or a weak suspension inoculated at several sites.

Another test was made one month later on June 21, using 10 trees for each point. Five spore loads were used per tree: 10, 10^2, 10^3, 10^4, 10^5. Each spore load was introduced in two ways: at one location, and divided among 10 locations in the tree. Data are given in Table 3.

TABLE 3. EFFECT OF MULTIPLE INOCULATION ON INFECTION BY DUTCH ELM DISEASE INOCULATED JUNE 21, 1944

No. of spores per tree	Number of trees showing wilt[1]		Average per cent wilt per tree		
	July 16	August 3	July 5	July 16	August 3
10	3	3	3.5	6.7	6.0
10 x 10	6	7	12.4	18.3	12.0
100	4	6	5.0	17.5	11.0
100 x 10	10	10	5.4	7.5	10.7
1,000	7	10	12.5	12.1	11.3
1,000 x 10	9	8	7.2	11.6	16.0
10,000	8	9	2.7	6.5	12.0
10,000 x 10	10	10	15.2	25.0	34.0
100,000	9	9	7.2	8.2	9.0

[1] Ten trees inoculated.

The second test confirmed the first in general, but since it was made one month later, it encountered the phenomenally dry weather of the summer of 1944 and, hence, fewer trees succumbed to inoculation. In general, multiple inoculation produced a larger percentage of diseased trees initially than a single inoculation and a larger percentage of affected leaves on the trees as well. It follows that many *Scolytus* beetles carrying a given spore load to a tree should provide a more effective inoculation and a wider involvement of the tree than one beetle carrying all the spores.

Effect of Age of Spores

Limited experiments have indicated that effectiveness of inoculation decreases with age of spores. This is confirmed by laboratory work on fungicides (6), which shows that as the age of spores increases they are less resistant to unfavorable factors in the environment and are easier to kill with fungicides. In June, 1944, elms were inoculated with spores 1, 2, 4 and 10 days old. Disease development was good on trees inoculated with younger spores, but was less on trees inoculated with the 10-day old spores. Again in July, five trees each were inoculated with spores 2, 4, 8, 16 and 22 days old. Disease symptoms appeared only in trees receiving two- and four-day old spores.

Part of this response may be the result of decreased viability of the spores with increasing age, but it is doubtful if this factor accounts for all of the variation.

FACTORS INFLUENCING PATHOGENICITY OF THE DISEASE IN INDIVIDUAL ELMS

Toxin Production by the Fungus

As the result of experiments conducted here, it has been shown that *C. ulmi* in culture produces a soluble toxic substance which is evidently the primary factor in the production of Dutch elm disease symptoms (39, 40). Previously, it was assumed that wilting and decline of diseased elms were solely the result of a reduced water supply brought on by plugging of the xylem vessels with gums and tyloses.

Toxin production was tested by growing the fungus for varying periods of time in liquid nutrient solutions (see Nutrition section), then filtering off the fungus mat by means of a Berkefeld filter, and injecting the sterile filtrate into small American elm trees, or testing its effect on various plant cuttings. Typical symptoms of the disease were reproduced in repeated trials when the filtrate was injected into elms; young leaves wilted, died and fell from the branches; older leaves curled upward or developed necrotic spots; walls of the xylem vessels became discolored, and gum plugs were formed in some vessels. These symptoms appeared within three days of the injection of 15 elms with from 75 to 200 ml. of toxic filtrate (from three-week old cultures) in 1941.

Sterile nutrient solutions adjusted to the pH of the filtrate from the fungus cultures were also injected into elms, with no effect beyond a slight discoloration of the wood near the point of injection. More typical disease symptoms were reproduced, however, when sterile nutrient solutions of lower pH (2.8 to 3.2) were injected.

Tomato, elm, maple and snapdragon cuttings have wilted severely when placed in tubes containing filtrates from fungus cultures growing under optimum conditions for toxin production. A common symptom produced on tomato cuttings placed in toxic filtrates, in addition to wilting, was a severe constriction of the lower stem. Occasionally, this occurred so soon after treating the cuttings that translocation of the toxic fluid to the leaves was negligible, and rapid wilting did not result.

Reproduction of disease symptoms by sterile filtrates from cultures of the fungus indicates that similar formation of toxins causes the disease symptoms in infected elm trees. Gums and tyloses are apparently also formed in response to the toxin which is produced and transported in the vascular system.

The relation of nitrogen source to toxin production was demonstrated by the following experiment. Three nutrient solutions were prepared: (1) KH_2PO_4 1.5 g., $MgSO_4$.$7H_2O$ 1.0 g., FeCl 0.01 g., glucose 25 g., yeast extract 2.0 g., and 1,000 ml. of distilled water; (2) same as No. 1 with the addition of 2 g. of asparagine per liter; (3) the same as No. 2 with the substitution of 2 g. of peptone for the asparagine. These solutions, in 250 ml. Erlenmeyer flasks, were inoculated with a heavy suspension of *C. ulmi* spores and tested for

toxin production by filtering off the fungus mats after 3, 6, 12 and 24 days growth and placing young tomato cuttings in the filtrates.

There was no evidence of toxin formation in the filtrate from three-day-old cultures, but some toxin had been produced by the sixth day in solutions (2) and (3) as three of five tomato plants wilted in the (2) filtrate and one out of five in the (3) filtrate. Results were similar with the filtrate from 12-day-old cultures.

In the filtrate from the 24-day-old cultures in (2) solution, five out of five tomato plants wilted severely within two hours. None of the plants in the other filtrates or in the nutrient check wilted until they had been in the solution six hours. After six hours, four of five plants in the filtrate from (3) solution were slightly wilted. Elm cuttings in the 24-day-old filtrates wilted overnight in both (2) and (3) filtrates, but not in filtrates from the (1) solution nor in control solutions.

Growth of the fungus was equally good on all three of the solutions described above, but toxin production was evidently favored by presence of asparagine. The toxin thus may be a product of the hydrolysis of asparagine, or may be a compound requiring asparagine or one of its degradation products for its formation. (In another test, toxic symptoms were obtained with the filtrate from a one-month-old culture containing asparagine as the nitrogen source, but were not obtained with a culture containing ammonium nitrate, and glycine.)

Little is known regarding the identity of the toxin or toxins. Extraction with toluol removes the toxic principle; it is also adsorbed on Norite (activated charcoal). It is thermostable to boiling for 10 minutes and to autoclaving for 20 minutes at 15 pounds pressure.

Plugging of the Vascular System

Plugging of the vascular system with gums and tyloses is another important factor affecting development of the disease. Apparently, it is a response to the toxin produced by the fungus, as mentioned in the preceding section. The brown discoloration seen on cutting a cross section of a severely diseased stem consists of large numbers of gum plugs in the xylem vessels and of brown stain in the xylem walls. Discoloration has been observed in vessel walls 20 inches above the inoculation point within 24 hours after inoculating trees with a heavy dose of *C. ulmi* spores, but gum plugs do not appear for several days. However, even before plugs appear in the wood, conduction of liquids is reduced as shown by experiments in which water was forced through stem sections at various times after inoculation.

In most of the experiments, stem sections of uniform length and as nearly as possible uniform diameter were connected to a six-inch glass funnel containing 500 ml. of water, by means of rubber tubing, and the amount of water passing through the sections in a given number of hours was recorded. Variations with different specimens

were large, but sections of stems from diseased trees usually permitted much less water to pass through than sections from healthy trees. In general, the resistance to water passage increased with the severity of the infection. The amount of plugging and discoloration in the wood also usually increased with severity of the infection.

In an experiment in June, 1941, 15 elms were inoculated with a spore suspension near the base. Five of these trees were cut at intervals of two, four, and eight days after inoculation and the water conductivity of comparable sections six inches long was compared to that of five healthy trees cut on each of the three dates.

Considerable variation was evident in transmission of water through sections from healthy trees on the different dates, but this was a paired experiment and in every case the water movement was less through the stems from diseased trees than in those from healthy trees. The variation in the sections from healthy trees evidently expresses the heterogeneity of the seedling population. Two days after inoculation the average amount of water passing through the sections from the inoculated trees in 15 hours was 75 ml., and the average for the healthy sections was 150 ml.; conduction of the diseased stems was thus only about 50 per cent that of the healthy. At four days the average amount of water forced through sections from diseased trees was 105 ml.; the amount forced through sections from healthy trees cut on the same day was 270 ml. Diseased stems, four days after inoculation thus permitted only 39 per cent as much water to pass as passed through healthy stems. After eight days only 55 ml. was forced through the diseased stems as compared to an average of 296 for healthy stems; conduction of diseased stems was only 19 per cent that of healthy stems. In another series, the amounts of water forced through diseased stem sections decreased with the number of days after inoculation as follows: 1 day, 115 ml.; 2 days, 65 ml.; 4 days, 75 ml.; 8 days, 25 ml., and 16 days, 20 ml.

Later comparative readings of water conductivity in other severely diseased and healthy stems also showed considerable differences, as indicated in Table 4.

It would be expected that, if plugging becomes a serious factor in reducing water movement through diseased stems, the efficiency of injection of elms with therapeutic chemicals should decrease with the length of time between inoculation and injection. An indication of this was obtained by injecting small trees with 8-hydroxyquinoline benzoate 1-1,000 one, two, four and eight days after inoculation. The trees injected only one day after inoculation were the least severely diseased by the end of the season; by that time there was little difference between treatments on the other three days. Trees injected one day after inoculation averaged 40 per cent wilt by the end of the season; those injected two days after inoculation averaged 54 per cent wilt; four days, 62 per cent wilt, and eight days, 54 per cent wilt, with 60 per cent wilt for the controls. Ten days after inoculation, the

TABLE 4. COMPARATIVE RATES OF WATER FLOW THROUGH DISEASED AND HEALTHY ELM STEMS

Stem section from:	Length of time diseased or since injection	Amount of water forced through stem section in: 6 hours	15 hours
Healthy tree	.....	150 ml.	.....
Tree injected with toxin	1 week	4 ml.	.....
Healthy tree	.....	.....	32 ml.
Tree injected with toxin	1 week	.....	2 ml.
Tree 70 per cent wilted	2 months	.....	1 ml.
Tree 35 per cent wilted	2 months	.....	35 ml.
Healthy tree	.....	.....	150 ml.
Tree 70 per cent wilted	3 weeks	.....	5 ml.
Healthy tree	.....	.....	75 ml.
Diseased tree	1 month	.....	5 ml.
Healthy tree	.....	.....	140 ml.
Tree inoculated in 1940, recovered, and cut in 1941	1 year	.....	160 ml.

effect of various times of injection could be more readily seen. Trees injected one day after inoculation averaged only 1.4 per cent wilt at this time, those injected two days after inoculation averaged 3 per cent wilt; those injected after four days averaged 7 per cent wilt, and those injected after eight days averaged 10 per cent wilt.

Condition of the Tree

Two different types of experiments have provided information on the relation of condition or general vigor of elms to development of the Dutch elm disease. One experiment involved a study of the effect of defoliation by cankerworms, the other, the effect of fertilization.

Effect of Defoliation

In the spring of 1944 many elms in an experimental planting at Mt. Carmel, Connecticut, were severely defoliated by cankerworms (*Alsophila pometaria* Harr. and *Paleacrita vernata* Peck). As other elms in the planting had been protected by lead arsenate spray, this provided an excellent opportunity for comparing development of the disease on defoliated with that on non-defoliated trees. On June 10, 20 elms from six to ten feet tall, which had been nearly 100 per cent defoliated, were inoculated in the top with a heavy dose of *C. ulmi* spores, and 20 non-defoliated trees were inoculated similarly. At this time the defoliated trees had not yet formed new leaves. On June 26, after the trees had refoliated, 20 additional defoliated elms were inoculated and compared with 20 normal, non-defoliated trees.

Disease development was markedly greater on the trees which had been defoliated by cankerworms than on those that had not lost their

leaves. As a result of the June 10 inoculations, the average percentages of crown wilted and die-back per defoliated tree by September, 1944, were 87 and 78 per cent, respectively, compared to 30 per cent wilt and 22 per cent die-back for non-defoliated trees. Results were similar following the June 26 inoculations, except that the disease did not progress as rapidly because of the later date of inoculation. Table 5 shows the results of this experiment.

The standard deviations of percentage of wilted foliage for both the defoliated and the non-defoliated trees which were inoculated on June 10 and 12 indicate considerable variation within treatments and the original data suggest that this was due to failure of a few trees to conform to the general trend. Percentage of die-back was quite uniform within the treatments. The deviation of individual trees in percentage of wilt and die-back for the later group of treatments was well within reasonable limits. It is therefore obvious that the mean percentage of wilt and of die-back was significantly much greater in both groups of defoliated elms.

TABLE 5. EFFECT OF DEFOLIATION BY CANKERWORMS ON DEVELOPMENT OF THE DUTCH ELM DISEASE

Type of tree	Date inoculated	No. of trees inoculated	No. showing wilt by 8/29/44	Average per cent disease per tree by 8/29/44	
				Wilt	Die-back
Defoliated	June 10, 1944	20	20	87±12.86	78±1.81
Non-Defoliated	" " "	20	20	30±17.04	22±3.27
Defoliated	June 26, 1944	20	18	28± 1.94	21±1.43
Non-Defoliated	" " "	20	13	11± 2.32	8±0.56

The marked acceleration of disease in defoliated trees may merely mean that in the process of refoliating, these trees are prolonging the spring growth period, which is a particularly favorable time for disease development. Another possible factor is inability of the defoliated trees to form some chemical which contributes to natural resistance of the elms to disease, i. e. "weakened" condition.

It seems likely that the increased disease development in elms which had been heavily defoliated by cankerworms explains in part the sharp increase in disease in local infected areas in Connecticut in 1944 (Table 8). In most of these areas the elms were heavily defoliated in both 1943 and 1944; many of them were defoliated twice each year, once by cankerworms and once by elm leaf beetles. On the contrary, the drop in number of new diseased elms in 1945 (Table 8), may have been due to the fact that cankerworms were only of minor significance in 1945.

Effect of Nutrition

In the experiment on effect of fertilization on susceptibility of elms to the Dutch elm disease, a nursery block of 50 elms ranging from six to eleven feet in height was divided into five randomized treatments of 10 trees each. Four of the treatments involved applying equal amounts of nitrogen, in the form of urea, ammonium sulfate, sodium nitrate, and 10–10–10 fertilizer; the fifth group of 10 trees was left unfertilized. Fertilizers were applied at the rate of 1/4 pound of nitrogen per inch of trunk diameter, in the spring and fall of 1942, and again in the spring of 1943. All trees were inoculated with a heavy dose of spores on July 7, 1943.

The results, as summarized in Table 6, indicate that disease appearance and development were appreciably less in trees which had been fertilized with sodium nitrate or with 10–10–10. When the results were analyzed in another way, by comparing disease development on trees in three vigor classes (vigorous, medium vigor, poor vigor), regardless of fertilizer treatment, an interesting relation was noted (Table 7). Fewer trees wilted and the progress of the disease

Table 6. Effect of Fertilization on Development of the Dutch Elm Disease

Fertilizer[1]	No. of trees treated	No. of trees in each vigor class at time of inoculation			No. of trees wilting	Average per cent disease (based on no. of trees wilting)			
						9/14/43		7/26/44	
		V[2]	M[2]	P[2]		Wilt	Die-back	Wilt	Die-back
Urea	10	3	4	3	8	64	56	68	60
Ammonium sulfate	10	1	8	1	10	50	41	55	52
Sodium nitrate	10	2	4	4	7	36	18	32	29
10–10–10	10	4	5	1	8	35	29	42	39
No fertilizer	11	1	5	5	11	51	41	52	47

[1] Each fertilizer was applied at the rate of ¼ lb. available N per inch of trunk diameter, on 6/12/42, 10/20/42, and 6/24/43. Trees were 6 to 11 ft. tall in 1942. Trees were inoculated with heavy dose of *C. ulmi* spores 7/7/43.

[2] V = vigorous, M = medium vigor, P = poor vigor.

Table 7. Effect of Vigor of Growth of Trees, Irrespective of Fertilization, on Development of Dutch Elm Disease[1]

Vigor class	No. of trees in each vigor class at time of inoculation	No. of trees wilting	Average per cent disease (based on no. of trees wilting)			
			9/14/43		7/26/44	
			Wilt	Die-back	Wilt	Die-back
Vigorous	11	6	19	15	27	20
Medium vigor	26	25	57	45	65	61
Poor vigor	14	13	42	34	45	43

[1] Trees were 6 to 10 feet tall when treated, and were inoculated in the top with a heavy dose of *C. ulmi* spores on July 7, 1943.

in those that showed symptoms was markedly less in trees which were classed as high in "vigor" (at the time of inoculation) than in those trees of poor or medium vigor. Criteria of "vigor" included size and color of foliage, diameter increment and shoot growth.

There were more vigorous trees in the fertilizer treatments than in the unfertilized group (25 per cent of the fertilized trees were classed as high in vigor, 9 per cent of the unfertilized); hence, it seems that fertilization had some beneficial effect in enabling the trees to withstand attacks of this disease. The trees which showed no disease symptoms in 1943 were re-inoculated in the spring of 1944. All showed symptoms to a varying degree, but the vigorous trees were not as severely affected as were the poorly growing trees in 1943. Further research is needed to clarify this problem, however.

The data so far in hand, however, are probably significant in the practical control of Dutch elm disease. It is well known that ornamental elms, especially street trees, exist in a low state of vigor as a result of the operation of civilization, sewers, water pipes, cuts, fills, curbs, pavements, etc. They are, therefore, probably more susceptible than forest and swamp elms.

Presumably, an important adjunct to control would be fertilization and watering of the trees.

Size of Tree

Disease appearance following artificial inoculation tends to become more erratic as the size of experimental trees increases. From the limited work done on this aspect of the problem, it seems that either an increased dose of spores or a greater number of inoculation points is necessary in order to obtain as much infection on larger trees as that obtained on small trees. As an example of this, only 22 of 32 large (18 to 30 feet tall) elms inoculated early in June, 1944, developed symptoms during 1944. All of 40 smaller (8 to 12 feet tall) trees inoculated at approximately the same time, with a similar spore dose, developed symptoms within three weeks.

Disease progress in a tree, once the infection is established, seems to be unrelated to tree size, however. Six elms five to seven feet tall were inoculated in the top on May 20, 1942; all six wilted, the average percentage of crown wilted by October 1 was 100 per cent, and the average die-back was 95 per cent. Six other trees seven to 10 feet tall, inoculated with the same spore dose on the same day all showed wilt, the average percentage of crown wilted by October 1 was 75 and the average die-back 60 per cent. Thus, the disease apparently proceeded down the stem at nearly the same rate in both groups of trees.

QUANTITATIVE ASPECTS OF DISEASE ADVANCE

In general, the advance of plant diseases through a population of susceptible hosts has been given remarkably little attention. Several

studies of the advance of Dutch elm disease have been made. For convenience the results will be discussed under three headings: (1) progress of the disease in an individual tree, (2) progress of the disease outward from an individual tree, and (3) progress of the disease in a geographical area with multiple sources of inoculum.

Progress of the Disease in an Individual Tree

As soon as an elm tree is inoculated through a wound, the spores of *C. ulmi* or its toxic excreta or both begin to travel through the vascular system. The movement of the spores or toxin through the tree has been investigated in the light of hydraulic theory which states that the movement of liquids through pipes is proportional to potential (inoculum potential in this case) and to time. Measurements were made, therefore, at successive time intervals following inoculation and for various spore loads. Data were taken on linear extent of vascular discoloration, water transmission by cut stems, and proportion of leaves wilted on the tree. It must be emphasized that methods were not available to measure directly the movement of either spores or toxins. Measurements could be made only on their effects. It is not even clear as to whether the effects are due to toxins only or to spores as well, but to avoid confusion on that point, the expression used will be "spores or toxin". The word, "or", is not expected to exclude the possibility that both may be present.

Progress of Vascular Symptoms

Data on water transmission and vascular discoloration were collected from an experiment begun on June 3, 1942, when 10 elms eight to 10 feet high were inoculated with spores at the base. Two trees were harvested at each of five time intervals. A few more trees would have been desirable. The amount of water that could be forced by gravity in 15 hours through the six-inch section above the point of inoculation was determined. The rest of the tree was dissected to determine the distance that discoloration extended. The effect of spore load was not determined in this experiment, which provides data only on time, not on potential.

The data were explored graphically. In order to have the curve on water transmission directionally similar to that for extent of discoloration, data on transmission were converted to reciprocals and expressed as resistance to transmission.

The data on reciprocal of water transmitted gave the best fit to a straight line on a log.–log. grid (Figure 4A). The same was true of the data on extent of discoloration except that the point for one day after inoculation was a gross misfit (Figure 4A). The conclusion is that the logarithm of time after inoculation is a linear function of the logarithm of the transmission of water (or resistance to transmission) and of the logarithm of the distance of discoloration. This conclusion may be confirmed by plotting logarithm of resistance to delivery against logarithm of distance of discoloration (Figure 4B), but again

the point representing discoloration one day after inoculation is a gross misfit.

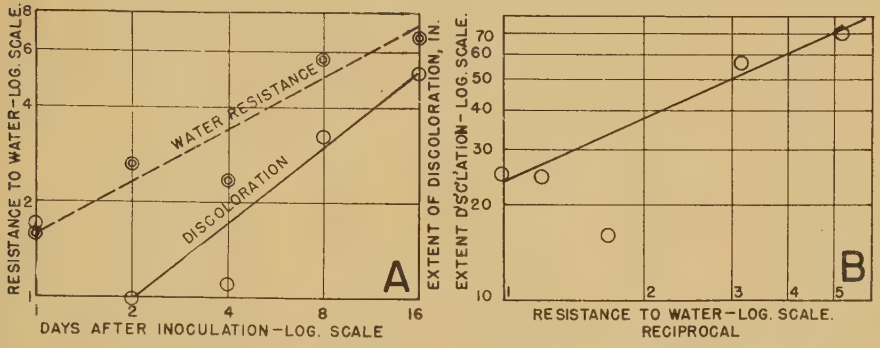

Figure 4. Quantitative studies on vascular symptoms of Dutch elm disease.

A. Effect of time on resistance to water transmission and on extension of vascular discoloration.

B. Relation between resistance to water transmission and extent of vascular discoloration.

Progress of Foliage Symptoms

The migration of spores or toxin was measured also by estimating the increasing percentage of wilted foliage as the season advanced. The effect of both spore load (potential) and time were included in an experiment on foliage wilting, the data for which have already been presented in Table 2 on page 15.

The data in Table 2 were explored graphically. It was soon apparent that time was related exponentially to the degree of wilting of the foliage for each of the spore loads. Accordingly, the data were plotted on logarithmic-probability paper (8) with time on the logarithmic x-axis and wilting on the probability y-axis (Figure 5). Fair fits were obtained for each of the different spore loads, especially for 10, 10^2, 10^3 and 10^5 spores. The fit for 10^4 and 10^6 were poor. The reason for the poor fits for these two spore loads is not clear. The points for 10^6 are all below expected values while those for 10^4 are simply heterogeneous. Inasmuch as the fits are poor, the points for the spore loads of 10^4 and 10^6 are omitted from Figure 5, in order to avoid cluttering the graph. The points may be inserted from Table 2, if desired.

Although there is a slight convex curvature to the lines, the agreement to a straight line is sufficiently close to draw the tentative conclusion that the response in probability of infection is proportional to the logarithm of time. This conclusion agrees with that that the response in vascular discoloration and resistance to transmission of water is also proportional to the logarithm of time.

Inspection of the data on spore load for each of the four readings during the summer suggests that spore load (potential) also bears a

logarithmic relation to probability of infection (Figure 6). A similar convex curvature shows, however, as in the time curves. The probable significance of the curvature will be discussed below.

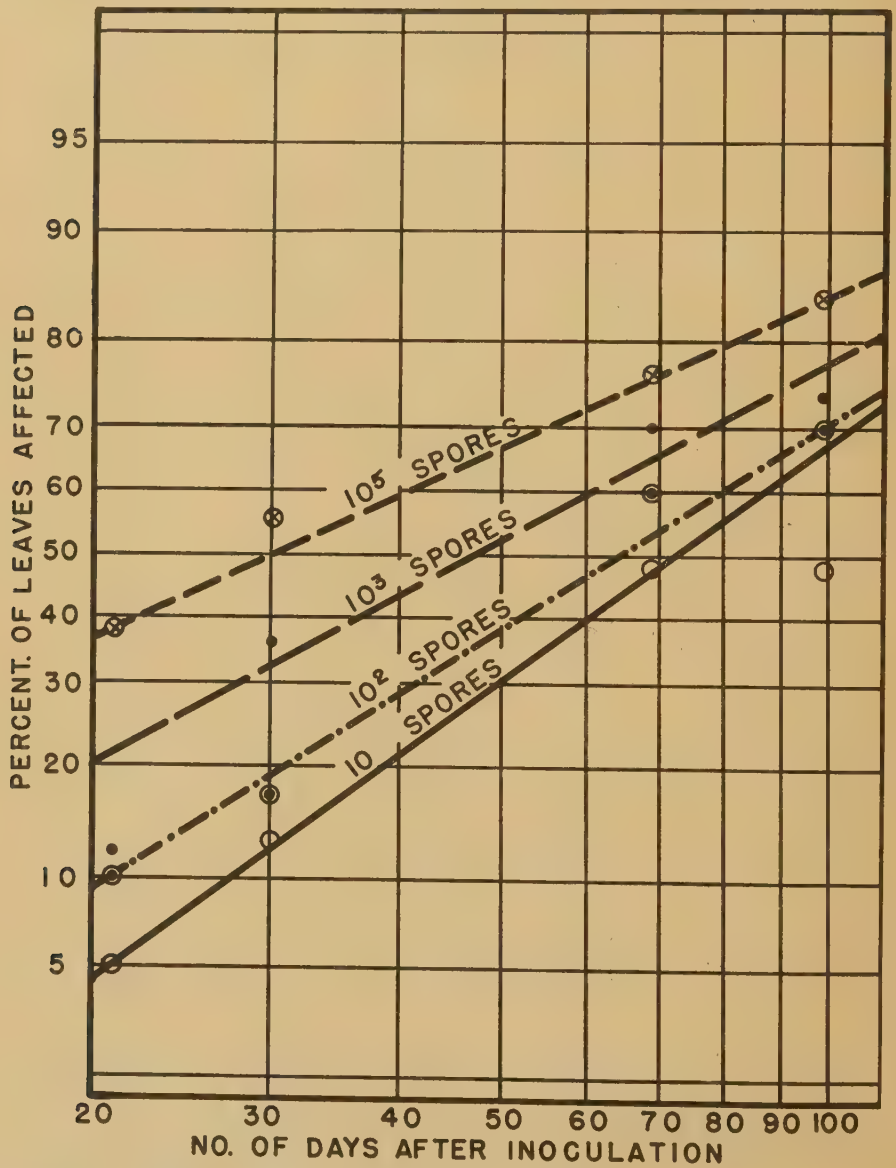

Figure 5. Effect of time on percentage of leaves wilted per tree by various spore loads.

Despite the curvature, it may be taken as the first approximation that the progress of the disease through the tree is proportional to the logarithm of the number of spores. Since the logarithmic effect of

time has already been indicated, it follows that spore load and time should bear a log.–log. relation to each other.

Therefore, interpolations have been made on Figure 6 for the number of spores necessary to produce 50 per cent of diseased leaves for the various observation dates expressed as number of days from

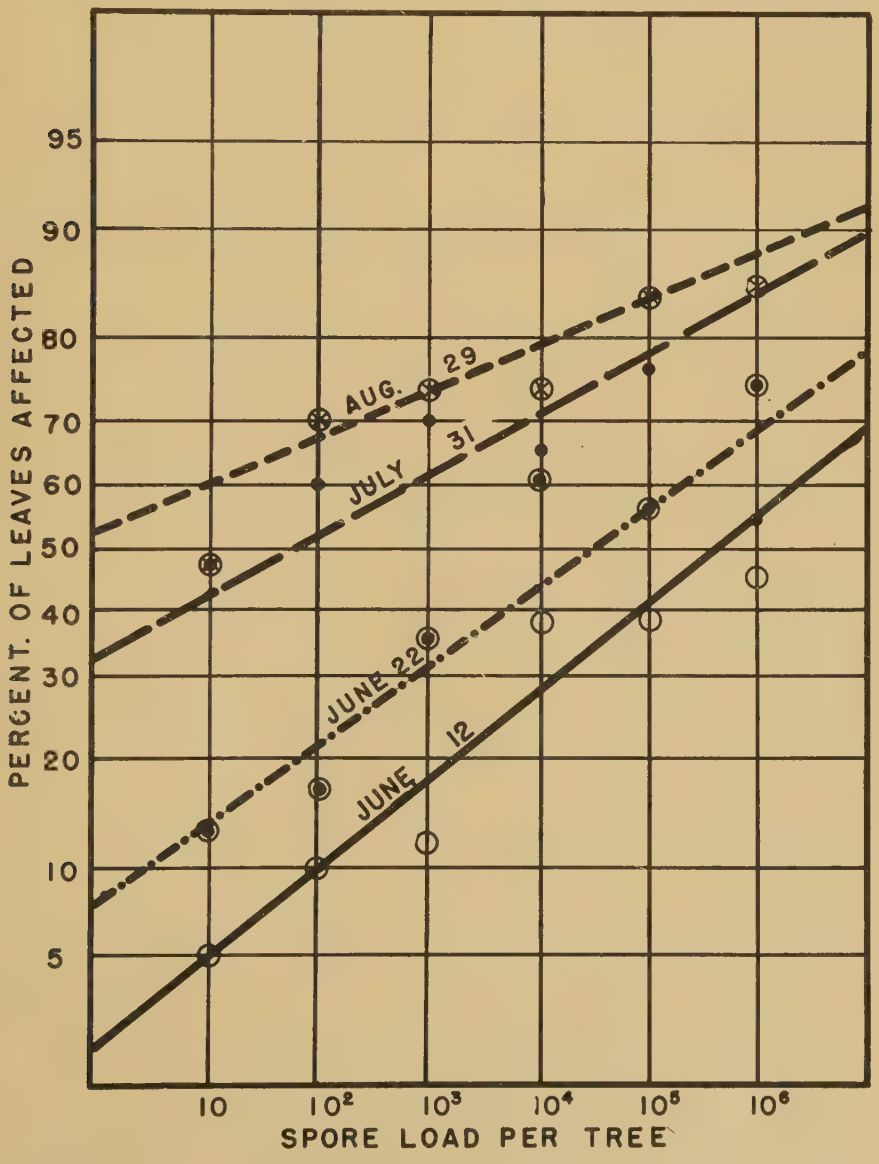

Figure 6. Effect of spore load on percentage of leaves wilted per tree on various dates.

inoculation. Results (crosses in Figure 7) are as follows: 316,000 spores for 21 days, 31,000 spores for 31 days, 40 spores for 70 days, and 2.5 spores for 99 days.

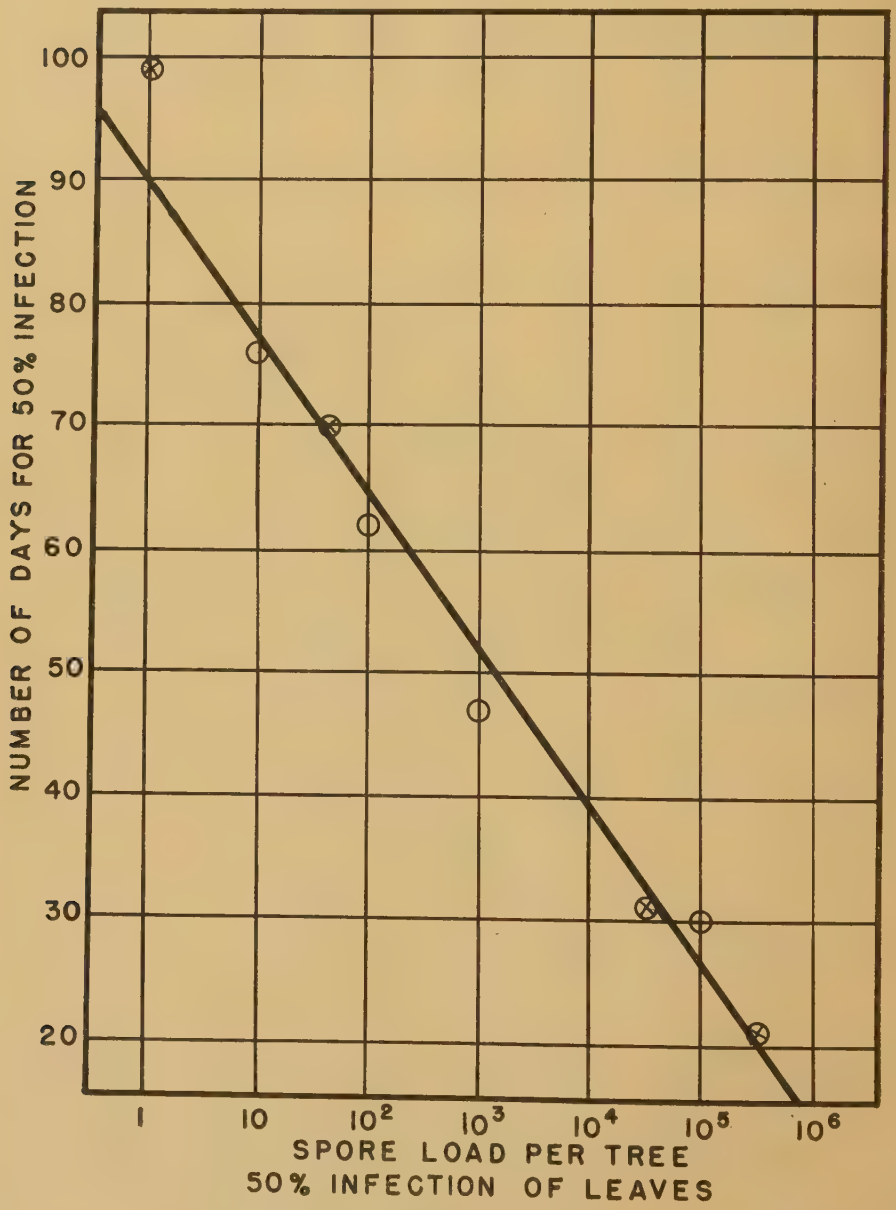

Figure 7. Relation between number of spores and time required to produce 50 per cent of wilted foliage.

Likewise, interpolations can be made on Figure 5 for the number of days taken for each of the spore loads to reach 50 per cent of diseased leaves. Results (circles in Figure 7) are as follows: 76 days for 10 spores, 62 days for 10^2 spores, 47 days for 10^3 spores, and 30 days for 10^5 spores.

If the results of the interpolations are plotted on log.–log. paper (Figure 7), it is clear that the curve is linear and this confirms the conclusion that the march of the disease through the tree is proportional to the logarithm of the number of spores to produce it and to the logarithm of the time in which the spores act.

It is interesting that the use of data for percentage of diseased foliage confirms the results from the use of vascular plugging as a measure. It is apparent immediately, however, that the conclusions are at variance with hydraulic theory of the effects of potential and time. The explanation seems obvious. The tubes in the elm stem do not remain constant in area, but they tend to become progressively plugged by tyloses and gums. In other words the fungus blocks its own path. The effect must increase exponentially with time and with potential (spore load).

The convex curvature in the lines for relating logarithm of spore load and also logarithm of time to foliage disease in Figures 5 and 6 cannot be ignored. Unless it can be explained adequately the tentative conclusions cannot be wholeheartedly accepted.

It must be remembered that distance is a hidden factor in the experiment on percentage of leaves showing disease symptoms. In order for additional foliage to be reached by the action of additional spores or additional time, the spores or toxin must travel through extra distance. Since distance bears a logarithmic relation to time, it means that the x-axis in Figures 5 and 6 carries a double exponent. The use of logarithms on the x-axis can correct for only one of these. The distance exponent that is left will help to produce the curvature.

The over-all conclusion must be that there is a proportionality between the progress of the Dutch elm disease through a tree and the logarithm of distance, logarithm of time, logarithm of spore load, and logarithm of resistance to water transmission. It is interesting that there is little apparent agreement between gradually plugging tubes and the idealized tubes dealt with in hydraulic theory.

Additional research is needed in this field, especially with toxins from culture.

Progress of the Disease Outward from an Individual Sick Tree

The natural spread of the Dutch elm disease into a population of hosts surrounding a sick tree is of considerable theoretical and practical interest. To obtain pertinent quantitative information on the matter, isolated, naturally infected trees were selected as centers for three plots in widely separated woodland and roadside areas in Fair-

field County, Connecticut (45). Long distance dispersal of contaminated beetles by wind may have led to infection of these isolated trees. The experimental design for these plots included the following primary requisites: (1) a single naturally infected tree at the center of each plot, as a source of inoculum; (2) an approximately random distribution of healthy elms in the vicinity; (3) separation of the plots from other diseased elms by at least one-half mile; (4) consideration of primary infections separately from secondary infections, so that a true picture of the pattern of spread could be obtained; (5) high level of *Scolytus* beetle infestation on diseased trees at plot centers, and (6) freedom of plots and immediate vicinity from beetle-breeding wood.

Figure 8. Diagram showing spread of Dutch elm disease from infection center (X). The diagram combines data from three plots; on each of them at "X" was an isolated, infected elm.

When first observed in the summer of 1941, each of the naturally infected elms at the center of each plot was severely diseased, and *S. multistriatus* adults were emerging from them. Some emergence undoubtedly occurred also during 1940. By the spring of 1942, all three trees had died from the Dutch elm disease. In this year also, emergence of beetles from secondary infections took place, so that disease appearance after 1942 could not be attributed to primary spread from the three original trees. The original diseased trees were 18, 38, and 24 inches in diameter on plots 1, 2, and 3, respectively.

During August and September, 1942, data were taken on new infections resulting from spread of the fungus from the original three trees. All elms within approximately one-quarter mile of the plot centers were examined for foliage symptoms of the disease; trees suspected of being diseased were sampled, and laboratory cultures were made. Composite results are diagrammed in Figure 8.

A preliminary inspection of the data showed that the disease decreased rapidly as distance from the original source of inoculum increased. The data were then classified into distance groups with means of 12.5, 50, 125, and 247.5 feet and plotted against the composite percentage of infection on logarithmic-probability paper (Figure 9). The relation was linear, showing that the probability of infection decreased with the logarithm of the distance from the source of inoculum.

Since the main mechanism of dispersion of inoculum is the flight and feeding of *Scolytus* beetles, the number of feeding injuries should show a similar decrease with distance from the source of beetles. Data of Wallace (33) and Wolfenbarger and Jones (38) indicate that just such an effect occurs.

The fact that probability of infection decreases with the logarithm of the distance suggests agreement with a similar relationship noted in the previous section on the progress of disease through the tree. The mechanism here is different, however. Here the flight of contaminated beetles occurs practically at random, at least it is certainly not channelized. As a result the beetles spread to all points of the compass, with the result that the chances of infection are spread over successively larger circles, and hence they fall logarithmically.

The data for the three spread plots and subsequent observations in a number of other localities provide useful information for estimating protective barrier zones in local control. From the regression line (Figure 9) plotted from data on the three local areas, it is apparent that when all probabilities are combined, the chances of infection for an elm within 25 feet of such large diseased trees were about 6 in 10. At 50 feet, the chances were about 3.5 in 10; at 100 feet, 1 in 10; at 300 feet, 1 in 100. By extrapolation it can be seen that the probabilities in infection at 500 feet were about 1 in 500, and at 1,000 feet approximately 1 in 10,000. It should be recognized that considerable

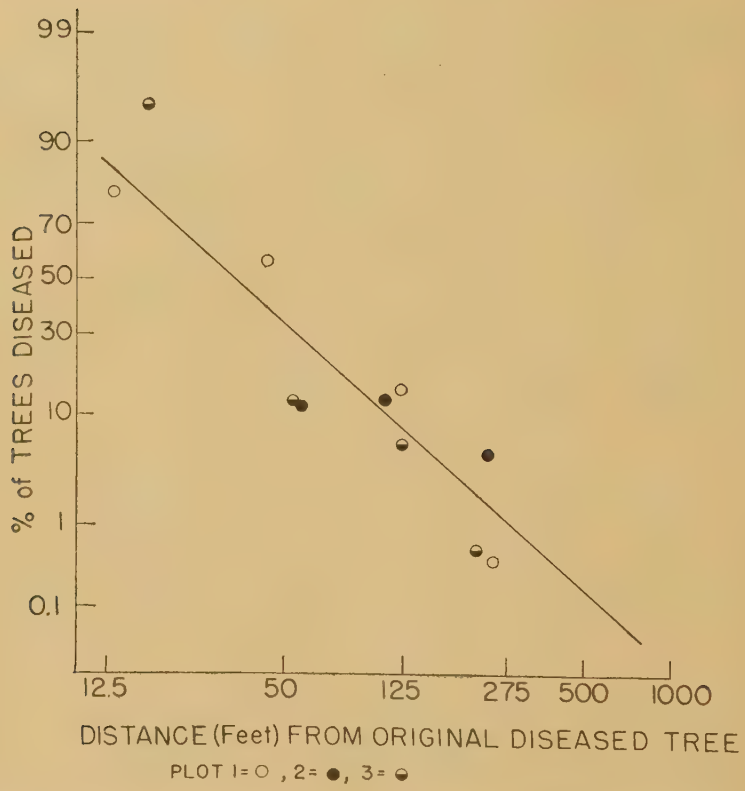

Figure 9. Regression curve calculated from data on local spread of Dutch elm disease from a single sick tree. Data were composited from the three plots.

variation in probabilities of infection can be expected with varying local conditions.

The impacts of the various factors on local spread are difficult to analyze. It is apparent that number, size, distribution, and condition of elms in a given area are important, as are also population of bark beetles, severity of infection, the spread of which is being studied, time of year of beetle emergence, time of felling and removal of the tree with relation to beetle emergence, presence of beetle-breeding wood, and weather conditions.

Data are lacking on many of these points, but some generalizations can be made. With only a few elms in the vicinity of an infection center, there should be a tendency for emerging beetles to migrate farther from the diseased tree in order to feed. With adequate numbers of elms in the vicinity, migration is restricted to relatively short distances. Size of surrounding elms should have ap-

proximately the same effect as number; large trees provide a larger target for the beetles. Distribution of trees is part of the same general picture; erratic distribution of hosts may lead to wider migration of the vector. Presence of suitable breeding wood is a very important factor (7), as beetles will migrate for considerable distances if such wood is not present near or in the tree or woodpile from which they emerge.

Condition of surrounding elms does not influence spread of the fungus directly, but influences the appearance of "spread". As noted above, trees which are heavily defoliated by insect pests or otherwise in poor vigor, develop more severe disease condition when inoculated with spores. Thus, a tree receiving a light dose of spores might develop symptoms only if in poor vigor.

From data on the season of most successful inoculation (p. 12) early season emergence of beetles is apparently the most critical. Late season infections frequently are of little consequence. Hence, removal of diseased trees before the emergence of beetles in May is of great importance. Unless diseased trees, which have been felled, are either removed or have the bark removed before beetles emerge, spread could conceivably be accelerated rather than lessened. Beetles which might normally feed in the still viable parts of the diseased tree and breed again in other dead and dying wood of the same tree would be forced to go elsewhere, with consequent spread of the fungus.

Size of the beetle population and its relation to severity of infection are also important factors. Wolfenbarger and Jones (38) presented data on influence of size of the beetle population. With five times as many beetles present, an increase in twig crotch injuries resulted; plotting these data on logarithmic-probability paper showed displacement of the regression line to the right when five times as many beetles were present, but the slope of the line was not affected. The severity of infection presumably governs the number of spores available for beetles to transmit. If the spore population were constant, presumably an increase in beetle population should result in greater spread of the disease, but again this is influenced by available hosts and other factors mentioned above. Weather conditions also play a part in the spread of this disease. Wind has been postulated as a factor in long distance spread of beetles (10), and seems to be a factor in greater spread in the north half as compared with the south half of the circular plots described above. Observations have already been noted on more rapid development of infections in wet seasons than in dry years. In addition to its effect on the growth of trees, precipitation may play a part in successful natural inoculation. Inoculation of elms when the branches are wet should provide a highly favorable condition for infection, as a spore suspension from the beetles' bodies should be sucked into the vascular system when beetles feed in twig crotches and expose vessels of the wood.

Progress of the Disease in an Area of Many Sick Trees

The study of the migration of Dutch elm disease around one sick tree is, of course, a simplified case. Actually, the disease in nature soon establishes multiple foci of infection. The action of the disease under such conditions can be studied best from the records of the United States Department of Agriculture Bureau of Entomology and Plant Quarantine which have been supplied to the Connecticut Agricultural Experiment Station as a cooperating agency.

It must be emphasized that such records are for trees found, not necessarily for trees actually diseased. The number of trees found depends upon the efficiency of the scouts in detecting the disease and on the amount of infection.

The records can be considered in two ways depending on the size of the area concerned: (1) a township or town as it is called in New England and (2) the entire state.

Just when the disease was introduced to the East Coast is not exactly known, but the point of introduction was probably New York City. In 1933 it was found in some quantity in New Jersey, in lower New York State, and in southwestern Connecticut. In 1934 scouting began in Connecticut and increased in intensity until 1939. In 1939 scouting was reduced somewhat in the early infected area of the State and was generally brought to a close there in 1940, but it has continued to date in those parts of the State at the margin of disease advance. Between 1934 and 1940 inclusive, all diseased trees found were removed.

Progress of the Disease in a Township

When federal scouting ceased in southwestern Connecticut in 1940, it became necessary to set up random samples there if the work on the progress of the disease was to continue. Accordingly, 20 plots were established (34, 35) in 1942 in Fairfield County which is the county in Connecticut closest to New York City. Five plots of one-quarter square mile area were randomly selected in each of four towns along Long Island Sound: Greenwich, Stamford, Darien and Norwalk. It must be emphasized that the plots are distributed over the whole town, not just in the urban area. The trees in the plots were examined in July and August in 1942 to 1945 inclusive. In 1942 all elms in each plot were counted and recorded. The number and percentage of diseased trees in each town could then be estimated.

The data on the advance of Dutch elm disease in Fairfield County are given in Table 8. Clearly, the advance of the disease through a population of elms can be considered as a species of growth curve. Fracker (11) so considered the advance of white pine blister rust and chestnut blight and he showed that "...the closeness with which infection percentages follow the Verhulst (36) population curve is almost startling". Barratt (4) suggested that growth of a leaf spot disease with time followed the probability function and Large (18)

has recently investigated both the logistic and probability functions in describing the advance of the late blight of potato. Large concluded that the two came so close that it would be impossible to distinguish them using ordinary experimental data.

Both have been investigated somewhat in connection with the advance of Dutch elm disease. As yet the data are insufficient to distinguish them. Since percentages of diseased trees are a function of probability of infection, the probability function has been chosen to represent this case. It is recognized, of course, that the percentages do measure growth of the disease, also, and that the logistic function may apply. Perhaps the true function lies somewhere between "logits" and "probits".

At any rate the data in Table 8 are plotted in Figure 10 on the basis of the probability function. It is evident that there is a linear relation with time on the x-axis and percentage of diseased trees (weighted for probability of infection) on the y-axis. In other words, the advance of the disease through the trees in an area is arithmetic with time.

The fact that time acts arithmetically is of considerable theoretical and practical interest. Normally, it would be expected that the number of beetles in a town would increase logarithmically with time because each female produces more than one offspring. The slope of the line would be expected to follow the mean number of offspring produced by each female. The probability of infection then would be expected to increase logarithmically with time but, of course, it does not.

It is known from other work on plant diseases, notably that of Heald (13) and McCallan and Wellman (22), that the probability of infection increases with the logarithm of the number of spores. That is because the spores compete with each other to produce infection. There is little reasonable doubt that the probability of infection by Dutch elm disease also follows the logarithm of the number of spores. Assuming an average number of spores per beetle, the probability of infection should follow the logarithm of the number of beetles.

Since several beetles may feed on a single tree, they compete with each other in infecting that tree. Therefore, even though the beetles increase logarithmically with time, the competition among beetles acts logarithmically in the opposite direction and the two logarithmic functions cancel each other, leaving the effect of time as arithmetic.

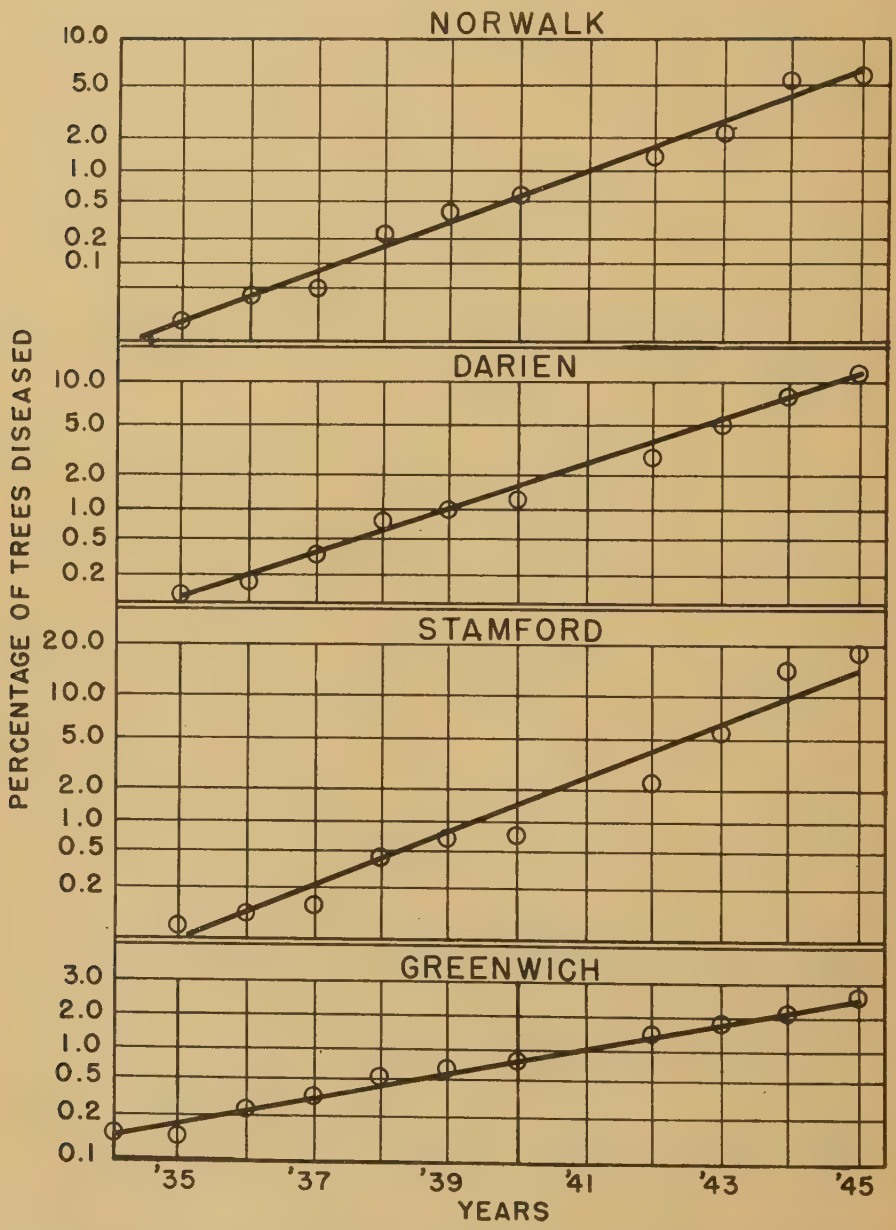

Figure 10. Progress of Dutch elm disease with time in four towns in Fairfield County, Connecticut. Data based on scouting reports supplied by United States Department of Agriculture from 1933 to 1940 and on random sample plots from 1942 to 1945. Percentages of infection in all cases calculated on basis of random sample plots.

The situation, perhaps, can be explained more easily by a series of algebraic equations. Let x = number of beetles, also number of spores, y = probability of infection, and z = time. The equation to describe a logarithmic curve for the effect of time on number of spores is:

$$\text{Log } x = kz + b,$$

where k is the slope of the line (growth rate) and b is the number of spores at zero time. Similarly, the equation for the effect of number of spores on probability of infection is:

$$y = K \log x + B,$$

where K is the slope of the line (growth rate) and B is a constant relating the probability of infection to spore population when the latter approaches zero. Substituting for log x, we have:

$$y = K(kz + b) + B,$$

which, of course, is an arithmetic function.

The mathematics of local intensification of the disease has been simplified for purposes of pedagogy by ignoring the beetle as an intermediary in the matter of spread but, of course, it cannot be ignored indefinitely. When "nature is in balance", the number of insects in an area appears to fluctuate around a mean. Nature is not in balance, however, in an area of increasing Dutch elm disease, where the amount of food materials for *Scolytus* beetles is increasing. We have no data on the subject, but presumably the beetle population increases to match the food supply.

Even if the total number of beetles does not increase in trees dying from Dutch elm disease, the number of *contaminated* beetles must increase because the proportion of contaminated food is increasing. Whether this increase in contaminated beetles is a logarithmic, probability or logistic function is, perhaps, academic, because the three functions would be exceedingly difficult to distinguish below 50 per cent disease with the field data available. If the function is indistinguishable from logarithmic, then the equivalence of beetles and spores is established for use in the algebraic equations discussed above, insofar as they apply below 50 per cent disease.

From the practical standpoint, the fact that the local intensification of the disease is arithmetic with time and not logarithmic means that the disease involves elms less rapidly than was feared at first.

The data in Table 8 and in Figure 10 are very useful in appraising the giant roguing experiment that was conducted during the 'thirties to control the Dutch elm disease. Of great importance is the fact that through 1940 every diseased tree found was removed. Since 1940 very few or no diseased trees have been removed from the plots. As a consequence, the portions of the curves prior to 1940 represent growth of the disease despite roguing out the sources of inocu-

TABLE 8. DISEASED ELM TREES FOUND IN FOUR TOWNSHIPS IN FAIRFIELD COUNTY, CONNECTICUT, 1934 TO 1945

Year	GREENWICH Number			STAMFORD Number			DARIEN Number			NORWALK Number			TOTAL Number		
	Annual	Cumulative	Per cent[1]	Annual	Cumulative	Per cent[2]	Annual	Cumulative	Per cent[3]	Annual	Cumulative	Per cent[4]	Annual	Cumulative	Per cent
1934	35	...	...	9	...	...	8	...	...	1	...	...	53	...	...
1935	34	69	0.127	27	36	0.08	6	14	0.10	4	5	0.02	71	124	0.08
1936	63	132	0.22	11	47	0.11	9	23	0.17	7	12	0.04	90	214	0.15
1937	63	195	0.32	27	74	0.17	24	47	0.34	2	14	0.05	116	330	0.23
1938	137	332	0.54	142	216	0.50	66	113	0.83	58	72	0.25	403	733	0.50
1939	80	412	0.67	100	316	0.74	29	142	1.04	41	113	0.40	250	983	0.67
1940	49	461	0.75	32	348	0.81	16	158	1.16	51	164	0.59	148	1131	0.77
1941[7]	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1942[5]	408	869	1.42	564	912	2.13	244	402	2.94	236	400	1.43	1452	2583	1.77
1943[5]	223	1092	1.78	1485	2397	5.60	292	694	5.08	283	683	2.44	2283	4966	3.41
1944[5]	223	1315	2.15	4006	6403	14.96	391	1085	7.94	851	1534	5.48	5471	10337	7.10
1945[5, 6]	482	1797	2.91	1115	7518	17.60	560	1645	12.05	402	1936	6.93	2559	12896	8.85

[1] Based on 61,215 elms in the town, estimated from sample plots.
[2] Based on 42,798 elms in the town, estimated from sample plots.
[3] Based on 13,664 elms in the town, estimated from sample plots.
[4] Based on 27,993 elms in the town, estimated from sample plots.
[5] Based on sample plots.
[6] The writers are grateful to Mr. John Schread for help in scouting in 1945.
[7] No records in 1941.

lum. Presumably, the portions of the curves since 1940 represent unobstructed growth of the disease in these areas. Had roguing been effective in checking the advance of the disease, it would be expected that the portion of the curve up to 1940 would show a slower rate of progress than the portion of the curve since that date. It is interesting to note that a single line seems best to fit the data for the whole 10-year period.

What evidence can be presented in favor of control by eradication? A detailed examination of the curves reveals what may be two significant irregularities in these curves: (1) the fact that for the years 1938, 1939 and 1940 the curves all tend to become very slightly flatter, and (2) the fact that from 1942 onward the curves may have become somewhat steeper in certain cases. How sound this evidence is depends upon the precision with which the actual amount of disease has been estimated by the methods used and upon the causes of these irregularities if they can be assumed real.

For the first irregularity, three possible explanations can be advanced: (1) The peak of the scouting effort was reached in 1938; reduced effort in 1939 and especially in 1940 may have resulted in failure to find all the diseased trees. This possibility is apparently negated by Brewer's (5) data on an area in New Jersey thoroughly scouted in all three years, for the data on such sample plots agree with the over-all data. (2) The roguing operations had begun to catch up with the disease and to reduce its rate of progress. (3) The weather factors may have been favorable to the disease during 1938 and unfavorable in 1940, thus causing respectively more rapid and more slow progress than on the average. In support of the third possibility is the fact that some of the points for 1945 are also below the line, yet roguing had not been practiced for five years.

The second irregularity points to the possibility that the curves have become slightly steeper since roguing was discontinued. However, the only curve for which this can be seriously considered is that for the town of Stamford; the other curves continue in trend unaltered.

Thus, the data presented suggest that the disease advanced just as rapidly prior to 1940 while roguing was in process as it did after 1940 when few or no trees were removed. One is tempted to speculate on the reasons for this. From Figures 9 and 10 it is clear that the disease spreads outward from diseased trees. Why, on the average, is this not prevented when the tree is removed?

Presumably, the individual tree must not be critically important in the whole picture of inoculum potential. Several explanations may be cited: (1) The tree is not removed until after the beetles have flown from it; (2) It is not completely destroyed, or rather the beetles in it are not completely destroyed; (3) Other sources of inoc-

ulum exist that are not destroyed, such as hanging dead limbs, elm woodpiles, dead bark patches on apparently healthy limbs. As Collins *et al.* (7) have pointed out and Rankin *et al.* (24) have reiterated, the supply of inoculum from such sources is tremendous and greatly exceeds that supplied by diseased elms. The presence of this reservoir of potential inoculum makes it difficult to alter the incidence of disease in large areas of woodland and swamp by removing occasional diseased trees. The cost of a program to clean up adequately such sources of infection would be astronomical. Local control in residential areas is a different matter, and it is apparent that the eradication program has resulted in some protection to elms in urban areas; (4) Lastly, there is the outside possibility that inoculum is directly transmitted without the need of beetles.

In no area of the country is it presently the custom to try to control the disease by roguing the whole population of elms. In several areas, especially in lower New York State, the effort is localized in the urban areas where scouting can be more intense and public cooperation is more easily enlisted. Officials there feel that eradication and rigid clean-up programs to remove beetle-breeding wood pay dividends.

The effect of the very removal of a diseased tree on the local spread of the Dutch elm disease is worth consideration. Normally, the emphasis has been to jerk out the sick tree at the earliest possible moment, with emphasis on *earliest*. When the tree is removed, it can no longer serve as a feeding station for contaminated *Scolytus* beetles. As a result the beetles that would have fed on it in the normal course of events must seek food elsewhere. Hence, they attack and sicken a new tree that might otherwise have escaped. The effect of this course of events is heavily exaggerated in case a few beetles escape from the tree before it is removed. As long as the tree is alive, it is the most accessible tree for the beetles emerging from it. In that sense the removal of a tree as long as it is not dead, encourages the spread of Dutch elm disease, perhaps as much as it discourages it.

The mathematics of the spread of the disease in a town suggest other practical matters. If the disease is a function of the logarithm of the number of beetles, and an effort is made to control it by removing beetles, then eradication of beetles must be much more efficient to produce a given effect than if the disease were an arithmetic function of number of beetles. Perhaps, the eradication efforts removed the competitive beetles. In any case, as the number of beetles is reduced by eradication methods, the efficiency of the eradication efforts must go up logarithmically if it is to produce unit reduction in beetle population.

In fact the tendency all during the period of roguing was to increase the efficiency of operation. First, the emphasis was on tree removal, then woodpiles were brought into the picture, and then hanging limbs. When it was found that the disease was spreading in

spite of all these improvements in technique, effort was shifted toward concentrating attention on limited areas that included only urban elms.

Progress of the Disease through the State

Moving from the town to the whole State, it is of interest to calculate and plot the migration of the disease through Connecticut. Assuming that the disease first entered the country at New York City, it probably migrated outward along radii from that city as a center.

A map showing the date of the appearance of Dutch elm disease in the various towns of the State appears in Figure 11. Neglecting the fact that a few of the radii pass over a portion of Long Island Sound, one can determine from that map the mean air-line distance between the towns and the Battery in New York City. If the towns are grouped by years, the mean cumulative distance of spread can be determined for each year.

When the data are plotted as in Figure 12, it is seen that the spread along a radius from New York City is linear with time and that the fit is remarkably good. If the disease continues to advance at its present rate, it should reach the northeast corner of Connecticut by 1950 $\pm$ two years.

At one time there was hope that the disease would decelerate when it reached the Connecticut River because the *Scolytus* population was smaller east of the river than west of it. Apparently, *Scolytus* is moving along with the disease, however, because the beetles have recently appeared in new towns east of the river.

It is interesting that here as with the Fairfield County data there appears to be no sign that the year 1940 marks any change in slope. That is, the disease seemed to advance just as rapidly through the State prior to 1940 as it did after 1940 and hence that roguing did little good in arresting its progress.

This is all hindsight insofar as the significance of roguing is concerned. Few, if any, felt in 1933 and 1934 that the roguing experiment should not be started. It was fairly generally agreed that no stone should be left unturned. The work was as well done as it could have been. If nature overrode the efforts, no one could be blamed.

It is of considerable scientific interest that the disease seems to advance across the State under conditions of multiple inoculations arithmetically in accordance with time. The situation is analogous to the growth of a fungus across a petri dish which is arithmetic with time. The reason is that any given hyphal tip grows a unit amount in unit time. Likewise, the mean maximum distance of beetle flight from a tree is presumably a constant. Beetles doubtless fly in all directions from a tree as indicated by data of Zentmyer

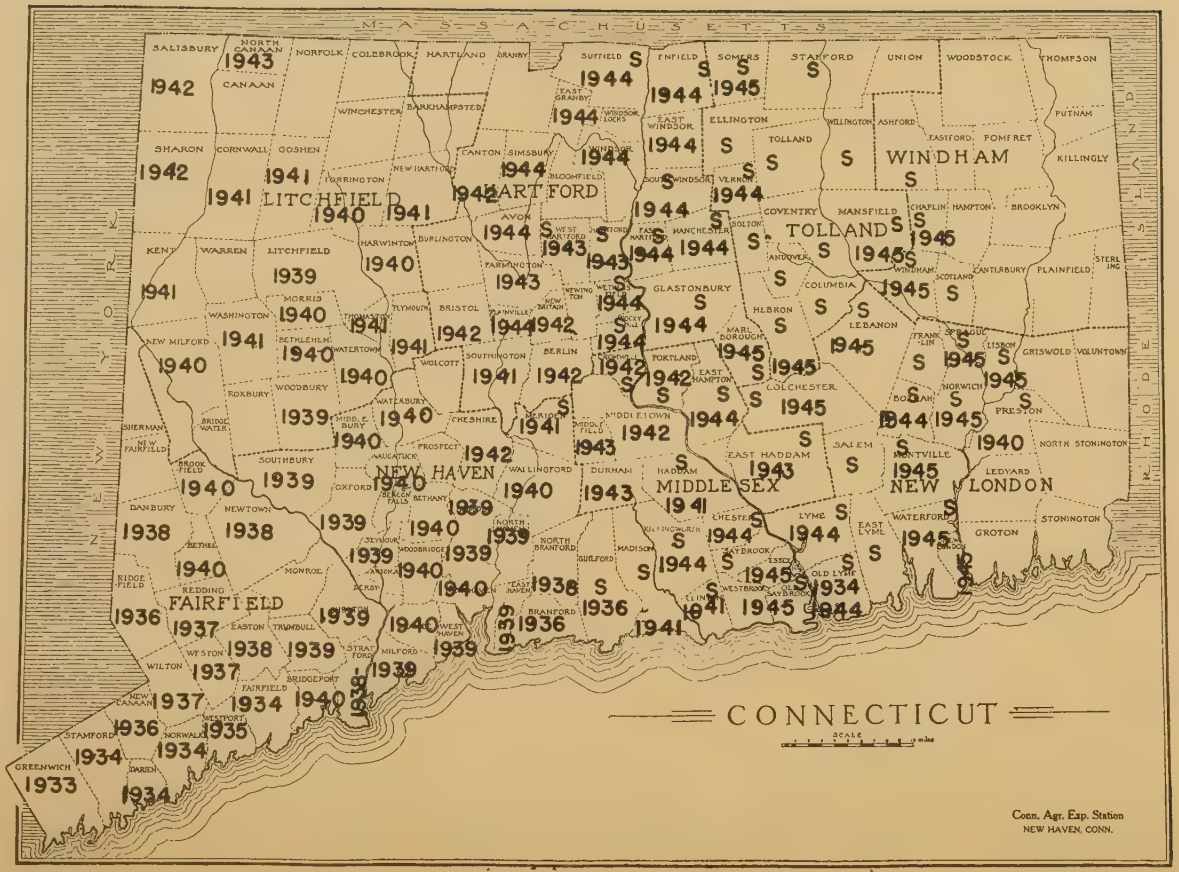

Figure 11. Date of appearance of Dutch elm disease in various towns of Connecticut. "S" means new occurrence of *Scolytus* beetles.

et al. (45) and Wolfenbarger and Jones (38). It is only the beetles that fly into a disease-free zone that carry the disease there.

The data provide some information on the mean maximum distance of beetle flight. The disease advanced from about 34 miles from New York in 1933 to about 99 miles in 1945 or 65 miles in 12 years.

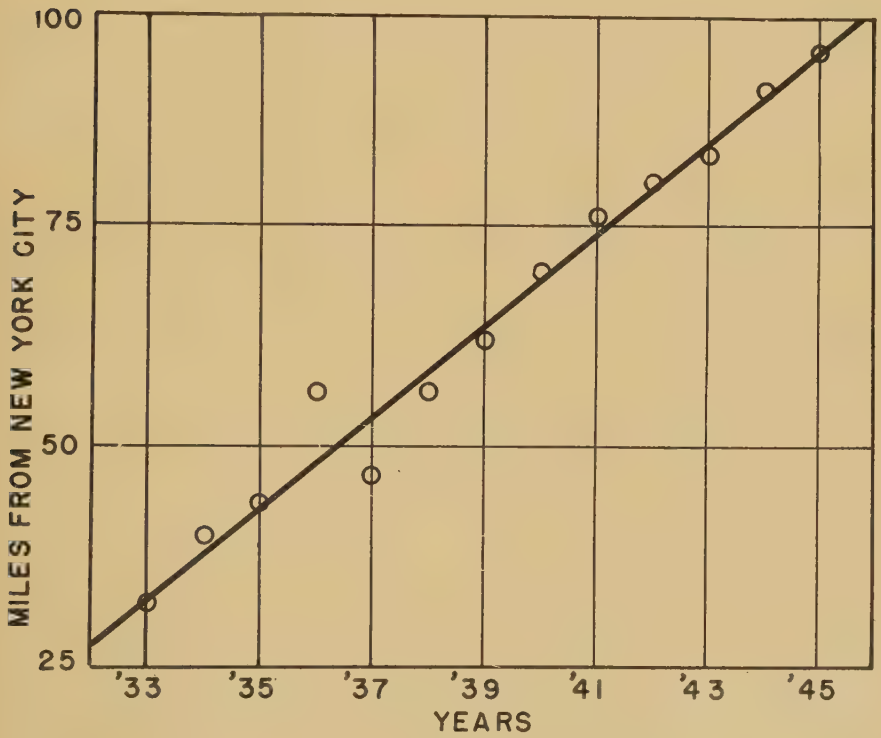

Figure 12. Effect of time on the march of Dutch elm disease across Connecticut.

This is a spread of 5.42 miles per year. Assuming that both generations of beetles fly equally as far on the average, the mean maximum distance of flight is about 2.71 miles.

Since prevailing air drift is northeastward, it is probable that, other things being equal, the disease is marching more slowly southwestward from New York than it is northeastward.

USING CHEMICALS INTERNALLY AS A METHOD OF DISEASE CONTROL

Using chemicals internally constitutes potentially the most effective approach to control of vascular diseases. Vascular fungi such as *C. ulmi* are inside the plant where they cannot be reached by the ordinary methods of spraying and dusting. The introduction of chemicals is, therefore, indicated.

Two of the three principles of plant disease control are involved when chemicals are used internally—immunization and therapy (14). Immunization is the principle of control in which a plant actively helps in warding off its enemies, or is assisted in warding off its enemies by application of chemicals internally. Theoretically, immunization is absolute, but for practical purposes nature is seldom absolute. It seems preferable to say that resistance to disease constitutes the grading of immunity.

Therapy is the principle of curing sick plants. Therefore, chemical immunization differs from chemotherapy chiefly in the matter of time. If the chemical is introduced into the plant before the fungus arrives, the principle involved is immunization. If the chemical is applied after the fungus has entered and produced disease, the principle is therapy. Chemotherapy has found successful application in controlling deficiency diseases, but to date has found no outstanding or widely accepted use against diseases caused by pathogenic fungi or bacteria. Stoddard (28) has recently reported significant results in preventing or controlling a virus disease by use of chemicals internally, however.

Experiments were begun here in 1940 to determine if the Dutch elm disease could be successfully controlled or prevented by introducing chemicals into the water system. The idea is by no means new, but many of the chemicals used and the concepts involved in approaching the problem are new (13, 14, 32). The aim has been not so much to kill the pathogen, as to reduce its pathogenicity so that the host is enabled to outgrow the infection. Of course a corollary to this approach is to keep the host healthy so that it can better withstand infection. Unfortunately, there is little knowledge of chemicals assisting in maintaining "health" of the tree, other than those used in commercial fertilizers plus added trace elements.

Effect of Chemicals on the Fungus

The chemicals on the opposite page were tested against *C. ulmi* in culture. Those reducing growth of the fungus by over 25 per cent at a concentration of one gram per liter are starred*.

These tests were made both by adding the chemicals to agar and by adding them to nutrient solutions. Two general groups of aromatic organic chemicals were found to be highly effective in preventing growth of the pathogen: the heterocyclic 8-hydroxyquinoline

Phenols
*Catechol
**o*-Nitrophenol
**p*-Nitrophenol
*Phenol
*Picric acid
*Pyrogallol
*Thymol
Hydroquinone
Resorcinol

Acids
*Acetyl salicylic acid
*Benzoic acid
**d*-1 Mandelic acid
*Salicylic acid
*Phenyl salicylate
Fumaric acid
Succinic acid

Aromatic Hydrocarbons
*Acenaphthene
*Diphenyl methane
*Fluorene
*Naphthalene
*Phenanthrene
Anthracene

Dyes
*Auramine
*Brilliant green
*Crystal violet
*Helione orange
*Helione yellow
*Neutral red
*Malachite green
*Methylene blue HCl

Quinine Alkaloids
*Cinchonidine HCl
*Cinchonine HCl
*Cinchonine salicylate
*Quinidine HCl
*Quinine bisulfate
*Quinine citrate
*Quinine HCl
*Quinine sulfate
Quinine salicylate
Quinisal
Cinchona alkaloids

Sulfa Compounds
**p*-Aminobenzene sulfonyl acetyl imide
**p*-Aminobenzene sulfonamide
**p*-Toluene sulfonamide
o-Toluene sulfonamide
Sodium sulfapyridine
Sulfathiazole

Quinolines
*Al 8-hydroxyquinolinate
*Ca 8-hydroxyquinolinate
*Mg 8-hydroxyquinolinate
*Zn 5, 7-dibromo-8-hydroxyquinolinate
*8-Hydroxyquinoline
*8-Hydroxyquinoline benzoate
*8-Hydroxyquinoline sulfate
*7-Chloro-2, 2', 4-trimethyl, 2, 3-dihydro-quinoline
*2-Hydroxyquinoline
Dimethyl quinolinate
6-Nitroquinoline
8-Nitroquinoline
Iso-quinoline
2-Hydroxy-8-chloro-4-methyl quinoline
2-Hydroxy-4-methyl quinoline
2-Hydroxy-4-chloro-methyl quinoline
2-Hydroxy-8-chloro-4-chloro-methyl quinoline
2, 2, 4-Trimethyl *o*-hydro-quinoline
8-Hydroxyquinoline 5-sulfonic acid
Zn 8-hydroxyquinoline 5-sulfonic acid
Quinoline
Quinolinic acid
Quinolinic diamide
Quinolinic imide

Quinones
*Chloranil
2-Methyl-1, 4-naphthoquinone
2-Methyl-1, 4-naphthoquinone diacetate
Quinhydrone
Quinone

Vitamins and Growth Substances
**p*-Aminobenzoic acid
*Naththaleneacetic acid
Ascorbic acid
Pyridoxine HCl

Miscellaneous N-containing Chemicals
*Acridine
*Arsphenamine
*Hydroxylamine HCl
*Mercaptobenzothiazole
*Salicylanilide
*Tetramethyl thiuramdisulfide
*Thiourea
**p*-Toluidine
Azochloramide
Chloramine T
Guanyl urea sulfate
Pyridine
Urea
Urethane

Miscellaneous Compounds
Formaldehyde
Potassium permanganate
Sodium thiosulfate

group and the triphenylmethane dyes. Several chemicals in these groups inhibited growth at dilutions of one to 50,000 or more.

The seven most toxic chemicals, in order of their toxicity (on a weight basis) to the Dutch elm disease fungus when added to malt extract agar, were: malachite green, 8-hydroxyquinoline sulfate, calcium 8-hydroxyquinolinate, brilliant green, 8-hydroxyquinoline, 8-hydroxyquinoline benzoate, and gentian violet. The chemicals were C. P. grade.

The effect of duration of exposure of the fungus to several of the more toxic chemicals was investigated, to determine if their inhibitory action was primarily "fungicidal" or "fungistatic" (21). Five-millimeter disks were cut from *C. ulmi* mats, placed in varying concentrations of the chemicals for one, five, 25 or 125 hours, washed in several changes of sterile water, and then transferred to test tubes containing nutrient media. Concentrations used were 1-1,000, 1-10,000, and 1-100,000 (with an additional concentration of 1-100 for 8-hydroxyquinoline sulfate). An exposure of 125 hours to a 1-1,000 dilution of 8-hydroxyquinoline sulfate did not kill the fungus; 25 hours at 1-100 resulted in death of the fungus, however. Another hydroxyquinoline derivative, 8-hydroxyquinoline benzoate was "fungicidal" only after 125 hours at the 1-1,000 dilution, as was the 8-hydroxyquinoline base. Malachite green was difficult to wash out of the disks, but still appeared to have definite fungicidal action after 25 hours at the dilution of 1-100,000. *p*-Nitrophenol was lethal after 125 hours exposure to the 1-1,000 dilution, as was juglone (5-hydroxy-1, 4-naphthoquinone) (12). Mercuric bichloride killed the fungus after only five hours exposure to the 1-1,000 dilution.

The theory has been advanced recently (42) that the 8-hydroxyquinoline group owes its inhibitory action to the removal of necessary trace elements from solution so that they are not available for the pathogen, thus blocking certain heavy metal enzyme systems. Laboratory work substantiates this theory. The use of these compounds in internal chemotherapy thus is apparently subject to the danger that they might also remove trace elements essential to the host.

Effect of Chemicals on the Fungus Toxin

In 1941 Howard (17) introduced a new approach to plant disease control by suggesting that the toxins formed by pathogenic fungi, specifically the bleeding-canker fungus on maple, could be antidoted by organic chemicals. A similar approach to the Dutch elm disease problem has shown that several organic chemicals will neutralize the effect of the toxin formed *in vitro* by *C. ulmi.* The wilting of cuttings has been prevented or reduced by adding 8-hydroxyquinoline sulfate to the toxic filtrate in a number of cases; urea, malachite green and orange helione (dihydrochloride salt of diamino-azo-benzene) have caused similar neutralization of the toxin (15, 16, 31).

Thus, it is evidently important in chemotherapy field trials to test chemicals which may affect the mechanism of pathogenesis of the fungus (by neutralizing toxins), or which may immunize the host, in addition to testing those compounds which are shown in the laboratory to affect the fungus directly. The types of chemicals which might be effective and should be given trials are thus greatly increased.

Effect of Chemicals on the Host

Chemotherapy and chemical immunization involve development of compounds which will not only prevent growth of microorganisms or prevent pathogenicity, but which will at the same time be relatively innocuous to host cells. With this double purpose in mind, most of the chemicals used in this investigation were organic.

Initial tests of the toxicity of approximately 50 chemicals to cut shoots of elm were made in the laboratory. Results are given in Table 9.

TABLE 9. PHYTOTOXICITY OF CHEMICALS AT 1-1,000 TO CUT ELM SHOOTS IN THE LABORATORY

No injury	Slight injury	Severe injury
p-Nitrophenol	8-Hydroxyquinoline sulfate	Thymol
Thiourea	Yellow Helione	Mercaptobenzothiazole
p-Toluidine	Orange Helione	*p*-Aminobenzene sulfonamide
Sodium thiosulfate	Chloramine T	*p*-Toluene
Oil cloves	Cinchonine salicylate	Quinhydrone
Oil cinnamon		Hydroquinone
Oil cedar		Carvacrol
Iso-amyl formate		Magnesium 8-hydroxyquinolinate
Sodium sulfapyridine		Aluminum 8-hydroxyquinolinate
Cinchona alkaloids		Zinc hydroxyquinoline-5-sulfonate
Cinchonidine HCl		8-Hydroxyquinoline benzoate
Cinchonine HCl		8-Hydroxyquinoline-5-sulfonic acid
Quinidine HCl		Chloranil
Quinine bisulfate		Salicylic acid
Quinine salicylate		Benzoic acid
Quinine citrate		Allyl isothiocyanate
Quinine HCl		Copper sulfate
Quinine sulfate		Arsphenamine
Quinisal		
Salol		
a-Napthaleneacetic acid		
Methyl salicylate		
Calcium 8-hydroxyquinolinate		
Zinc 5, 7-dibromo-8-hydroxyquinolinate		

Some of these chemicals were carried to the field in 1940 and their toxicity to elms was tested by injecting trees two to six inches in diameter (12-30 feet tall) in swamp and woodland areas near New Haven. Seventy-eight trees were injected with several concentrations of 23 different chemicals (Table 10). Uptake of the solutions

was good with 8-hydroxyquinoline sulfate, gentian violet, resorcinol, sodium thiosulfate, copper sulfate, and borax, applied through holes bored in the trunks. Commonly, one to two gallons of these solutions were taken up in 24 hours during June and July, by trees 15 to 20 feet in height. Slight injury was noted with 8-hydroxyquinoline sulfate at a concentration of two grams per liter; some necrosis of leaf tissue and discoloration of wood occurred. One gallon of thiourea at a concentration of two grams per liter caused appreciable injury on trees 12 to 15 feet tall, as did one gallon of sodium thiosulfate at the same concentration.

The two materials giving most severe injury were copper sulfate and borax. Two elms 14 and 15 feet tall were each injected, through a single bore hole in the lower trunk, with one gallon of copper sulfate on August 29, 1940. One tree received a solution with a concentration of two grams per liter, the other a concentration of one gram per liter. By September 2 the solutions had been entirely taken up, and 80 per cent of the leaves had fallen on the tree receiving the two grams per liter solution; 50 per cent of the leaves had fallen on the tree receiving one gallon of one gram per liter concentration. By September 5 all leaves had either been killed or had fallen from both trees. By early October both trees were dead.

Boron injury was apparent within a week on trees injected in the trunk in June 1940, with solutions of borax. Trees 15 to 30 feet in height were injected with one gallon of solution containing either 28, 14, 7.5, or 3.7 grams, and with two gallons containing 28, 14, 7.5 or 3.7 grams. Some of the trees took up two gallons in five to six days, one gallon in two days. In three weeks, with the higher concentrations (28 or 14 grams per gallon), all leaves on the trees were killed. Injury was severe with all treatments except one gallon containing 3.7 grams (1-1,000 conc.) Twigs and larger branches were killed back extensively.

In another experiment elms six to 30 feet in height received dry applications of borax in the root zone; four trees each were given two, one, one-half, and one-quarter pound applications. Injury appeared about a month after treatment and consisted of severe leaf burning and twig die-back on smaller trees (8-10 feet tall) receiving two pounds and one pound of borax. No injury occurred with the one-half or one-quarter pound applications, nor on the larger trees with two or one pounds. In another experiment 125 grams of borax were applied dry in the root zone of 10 elms four to six feet tall. All trees were so severely injured by the chemicals that disease symptoms could not be distinguished from subsequent inoculations.

TABLE 10. PHYTOTOXICITY TESTS WITH ORGANIC CHEMICALS
Effect of Injecting Organic Chemicals into Elm Trees 12 to 30 Feet in Height

Chemical	No. of trees injected	Uptake	Notes on injury
Acetylsalicylic acid	1	Fair	Slight injury at 1-1,000 conc.
Borax	8	Good	Severe injury with 1/2 oz. in 1 gal. water, with 1 oz. in 1 gal., with 2 gals. of 1-500 conc., with 2 gals. 1-1,000 conc. None with 1 gal. 1-1,000.
Catechol	1	Fair	None with 1-1,000, 1 gal.
Copper sulfate	2	Good	Trees 2" in diam., 15 ft. tall killed with 1 gal. 1-500 conc. and 1 gal. 1-1,000.
Chloramine T	2	Fair	None with 1 gal. 1-500 or 1-1,000.
Gentian violet	2	Good	None with 1 gal. 1-5,000.
Orange Helione[1]	3	Fair to poor	None with 1 gal. 1-500.
Yellow Helione[1]	4	Very poor	None. Little uptake whether trees bored under water or not.
Hydroquinone	1	Fair	None with 1 gal. 1-1,000.
8-Hydroxyquinoline	2	Fair	" " " "
8-Hydroxyquinoline sulfate	20	Good	Slight injury with 1 gal. 1-500; no external injury with 1-1,000 conc.–slight wood stain.
8-Hydroxyquinoline benzoate	7	Fair	Slight injury with 1-250 and 1-500 concs.
Potassium permanganate	2	Fair	None with 1 gal. 1-500 or 1-1,000.
Resorcinol	2	Good	" " " "
Quinine sulfate	2	Fair	" " " "
Salicylanilide	2	Fair	" " " "
p-Aminobenzene sulfonamide	2	Poor	" " " "
p-Toluene sulfonamide	7	Fair	Moderate injury with 1-500 conc.
Sodium sulfapyridine	2	Fair	None with 1 gal. 1-1,000.
Sodium thiosulfate	2	Good	Considerable injury with 1 gal. 1-500.
Thiourea	2	Fair	" " " "

[1] Dihydrochloride salt of di-amino-azo-benzene.

From 1940 to 1944 approximately 1,100 nursery elms (four to 12 feet in height) were treated with 37 different chemicals as follows:

Di-*n*-octyl amine
Acridine
Ascorbic acid
Auramine O
*Benzoic acid
p-Aminobenzoic acid
*Cupferron
Disodium ethylene bis-dithiocarbamate (Dithane)
*Fumaric acid
*Hydroquinone
*Hydroxylamine HCl
Yellow Helione
*Malachite green
Mercaptobenzothiazole
Magnesium sulfate
Methylene blue
Neutral red
**p*-Nitrophenol
Phenothiazone
Potassium chloride
Potassium nitrate
Potassium permanganate
a-Benzyl *a*-phenylhydrazine HCl
*Pyrocatechol
Pyrogallol
Quinhydrone
8-Hydroxyquinoline
8-Hydroxyquinoline benzoate
*8-Hydroxyquinoline sulfate
Quinone
Quinine HCl
Tetrachloroquinone (Spergon)
Tetramethyl thiuramdisulfide
*Sodium thiosulfate
Thymol
Urea
Vanillin

Chemical injury was not common; most of the chemicals were used in injection safely at a concentration of one gram per liter, in amounts varying from 100 to 1,100 ml. per tree. Any chemical showing more or less injury is starred*.

In addition, approximately 30 larger trees (15 to 70 feet in height) were injected with 8-hydroxyquinoline sulfate and benzoate, and hydroquinone. One large tree received 43 gallons of 8-hydroxyquinoline sulfate solution: 33 gallons at a concentration of one gram per liter and 10 gallons at two grams per liter. Injections in the trunk were made at intervals from June 28, 1940, to September 5, 1940, and again on May 26, 1941 and June 2, 1941. No chemical injury was apparent. The tree was naturally infected with *C. ulmi* and, after injections were started, disease progress appeared to be appreciably retarded during 1940 compared to other trees of similar size and involvement. However, in 1941, leaves were small, wilting increased rapidly and the tree died in July. No injury was apparent either on five trees 18 to 30 feet in height, all of which took up either one or two gallons of 8-hydroxyquinoline sulfate in two to three days.

Hydroquinone caused no injury when injected into large elms, at a concentration of one gram per liter or 0.67 gram per liter. A tree 22 inches in diameter was injected, through four holes bored in the lower trunk with four gallons of hydroquinone (0.67 grams per liter) in the morning of July 20, 1941. Four hours later all four gallons had been taken up in the vascular system, and four more gallons were applied. The solution was completely taken up by the following morning. No injury was evident.

There is, of course, a possibility with all of these organic chemicals of injury to elms or other hosts from accumulation of injurious

amounts of the chemical in the leaves. This may result from application of a large volume of solution of relatively low concentration, or a small volume of solution of high concentration. Most of the organics, however, are far less toxic to plants when applied internally than are some of the inorganic chemicals—particularly the heavy metals.

Effect of Chemicals on the Disease in the Field

As mentioned in the previous section, approximately 1,100 nursery elm trees, ranging from four to 12 feet in height, have been treated with chemicals in various ways in attempts to prevent or cure the Dutch elm disease. Most of the work involved injection directly into the lower trunk, on trees artificially inoculated with *C. ulmi*, and growing under closely comparable conditions. A number of larger trees, both artificially and naturally inoculated, were also treated.

In the injection experiments the gravity method was used exclusively. Two main types of injections were tried. In the first, a hole (or holes) was bored near the base of the trunk with a brace and bit or hand drill, a piece of tapered brass tubing was driven into the hole or a threaded piece of tubing screwed into the hole, and this tubing was connected by rubber tubing and glass connections to a suitable reservoir (flask, gallon jug, beer can) suspended above containing the chemical to be injected. Holes were bored in such a manner as to contact as much sapwood as possible. The second method was developed by E. M. Stoddard in chemotherapy work on viruses at this Station, and involves cutting off a terminal or side branch, and attaching this to the reservoir of chemical solution by means of rubber tubing. In another type of injection, holes of various sizes were bored in the trunks to accommodate various sizes of gelatin capsules containing water-soluble chemicals in dry form. Capsules were inserted, water pipetted in to fill the holes almost completely, and corks were driven in to close the cavities. This is a convenient type of injection as the water for diluting the chemicals is supplied by the vascular system; considerable, but not serious, local injury results at the site of injection. As a variation on this method, measured amounts of dry chemicals were forced into holes bored in the trunk, then water added and the holes closed as above; the only difference being in the omission of the gelatin capsule.

Applications of chemicals, either in dry form or in aqueous solution, to the soil, in basins at the base of the trees, have also been used here extensively. Spraying of chemicals on the foliage of elms has been given only limited trials, but deserves further investigation as a chemotherapeutic measure. May (19) has described a number of methods of tree injection.

Large amounts of chemical solutions can be introduced into trees by the bore-hole method, but injury to an often already weakened

vascular system is an undesirable feature of this type of treatment. For putting chemicals into the tops of trees, the method of application through cut ends of branches is more satisfactory. However, soil applications seem to offer even greater promise and have the very definite advantages of obviating injuries to the trunk or branches and better distribution of materials when taken up by the root system. Soil applications and possibly spraying of foliage seem to be the most promising avenues of approach to control or prevention of vascular diseases by use of chemicals internally.

Immunization

In a number of trials, trees were injected, or treated by one of the other methods mentioned above, prior to receiving a severe inoculation with *C. ulmi* spores. The object was to determine if sufficient amounts of chemicals could be introduced into the vascular system to prevent, or retard development of the disease. In this work most of the nursery elms were treated with chemicals one week or ten days before inoculation with the fungus; some were injected a month before inoculation. Trunk injections and soil treatments will be discussed separately.

It should be emphasized that plants have only limited means of showing responses to experimental treatments. In these experiments wilting and death of foliage were the criteria used. Wilting and death can be due to the chemicals used as medicines as well as to the disease that they are designed to treat.

The first injection work was done in 1940 when 264 small elms were used. Injection of approximately 250 ml. of 8-hydroxyquinoline sulfate per tree two weeks before inoculation on August 13 showed an accelerating effect on development of the disease while injection of the same chemical at the time of inoculation retarded appearance of disease, and injection two weeks after inoculation had little effect (39, 41). Flasks containing 250 ml. of 8-hydroxyquinoline sulfate at a concentration of one gram per liter were attached to each small tree. Twenty-nine of the 50 trees injected July 31-August 1 took up the entire 250 ml. within one week; the average amount taken up by all 50 trees in nine days was 216 ml. Fifteen trees showed slight to moderate leaf injury. Thirty-five of 54 trees injected August 13 took up 250 ml. in one week; the average amount taken up by the 54 trees in nine days was 236 ml. Seven of these trees showed severe injury, 40 showed slight to moderate injury. Twenty-eight of 55 trees injected August 26 took up 250 ml. within one week; the average amount taken up in nine days was 216 ml. Thirty-six trees showed slight to moderate injury.

The injections on July 31-August 1, two weeks before inoculation, may have caused a more severe disease condition for two reasons: first, because of the injury to the vascular system by boring a hole in the stem, and second, because most of the chemical may have accumulated in the leaves after two weeks, leaving very little in the

vascular system to affect the fungus. In the injections at the time of inoculation, disease progress was definitely retarded, with only 11 per cent of the trees showing disease symptoms by August 26, contrasted to 27 per cent of the control trees. By the end of the season, 59 per cent of the trees injected at the time of inoculation had developed symptoms, while 75 per cent of the control trees were wilted. Apparently, by injecting the trees at the time of artificial inoculation, sufficient chemical was present in the vascular system to retard development of the fungus, and thus to counteract any deleterious mechanical effect of the injection method. Injection two weeks after inoculation had little effect because the fungus had become well established in the small trees. The season of 1940 was wet; consequently, infections developed well even in August.

With small trees (three to six feet tall) a marked protective effect was obtained with four out of eight organic chemicals injected through holes bored in the bases of the trunks, in June, 1941 (32). Beer cans, containing 250 ml. of the solutions, were connected to pieces of brass tubing inserted in the injection holes. Hydroquinone, *p*-nitrophenol, benzoic acid, and 8-hydroxyquinoline sulfate markedly reduced the percentages of trees becoming diseased, following inoculation in late June, 1941 (one week after injection). Table 11 presents data on this series. None of the five trees injected with *p*-nitrophenol at a concentration of one gram per 1,500 ml. became diseased,

TABLE 11. EFFECT OF INJECTION OF SMALL ELMS WITH ORGANIC CHEMICALS PRIOR TO INOCULATION WITH *C. ulmi*, IN 1941[1]

Chemical	Concentration	No. of trees injected	Average amount of solution taken up per tree	No. of trees wilting	Percentage of trees wilting
p-Nitrophenol	1-1,500	5	180 ml.	0	0
p-Nitrophenol	1-1,000	5	165	5	100
Hydroquinone	1-1,000	8	240	1	12.5
Benzoic acid	1-1,000	9	175	4	44.5
Hydroquinone	1-1,500	5	230	3	60
8-Hydroxyquinoline sulfate	1-1,200	10	210	6	60
8-Hydroxyquinoline	1-1,500	13	185	11	84.5
8-Hydroxyquinoline	1-1,000	9	135	8	89
Thymol	1-1,000	13	150	13	100
Potassium permanganate	1-1,000	5	170	5	100
"Helione orange"[2]	1-500	5	110	5	100
Control—water injected	...	10	110	10	100
Control—uninjected	...	6	...	6	100

[1] These trees were all injected at the base and inoculated with a heavy spore suspension in the trunk, about two feet above the base. Trees were from 3-6 feet tall.

[2] Dihydrochloride salt of di-amino-azo-benzene.

while all of the trees injected with *p*-nitrophenol 1-1,000 showed symptoms, which is difficult to interpret. Only one of eight trees injected with hydroquinone at a concentration of one gram per liter became diseased; the lower concentration (1-1,500) did not have such a striking effect, three out of five trees becoming diseased. Benzoic acid prevented disease development in five out of nine trees, 8-hydroxyquinoline sulfate in four out of ten trees. All of the 18 untreated control trees became severely diseased.

Results in 1942 were not as striking as in 1941. Fifteen different chemicals were injected into the bases of trees from six to 10 feet tall, on July 7 and 8 and inoculated in the tops about five feet up on July 17 and 18. The percentage of foliage wilting was read periodically during the season. Whether injected or not, practically all trees showed wilt during the season (Table 12), in contrast to the results in 1941.

The data have been evaluated like those on pages 29 and 59 with Barratt's (4) new method. Infection data were plotted against time on a log.-probability grid and the best straight line was drawn by eye. Slight curvature discussed on p. 29 was ignored when encountered. From the curves can be taken the number of days to produce a given level of disease (say 50 per cent).

Examination of the data in Table 12 show that the march of disease through the tree is slowed by 8-hydroxyquinoline sulfate, hydroquinone, quinone, *p*-nitrophenol, and probably by pyrogallol and pyrocatechol. No effect was observed with vanillin, hydroxylamine hydrochloride, 8-hydroxyquinoline benzoate, sodium thiosulfate, quinhydrone, methylene blue and benzoic acid. Perhaps potassium permanganate was injurious.

Soil treatments were tried in 1941, 1944 and 1945. In 1941, they showed variable results. Thirty trees watered with 8-hydroxyquinoline sulfate (at two grams per liter), several times before inoculation on August 11 and again after inoculation, wilted more severely than 30 trees around which water alone was applied. These trees (three to five feet tall) each received 8½ gallons of solution. Watering immediately after inoculation on August 11, and several times thereafter, in another experiment, resulted in an average wilt of 13 per cent for 10 trees receiving five gallons of 8-hydroxyquinoline sulfate (two grams per liter), compared with 32 per cent wilt for 10 trees watered with five gallons of hydroquinone (one gram per liter), 33 per cent wilt for 10 trees watered with five gallons of benzoic acid (one gram per liter), and 39 per cent for controls receiving five gallons of water. In a third experiment, watering two weeks after inoculation with spore suspension on July 31 resulted in no significant difference in disease development on trees treated with 8-hydroxyquinoline sulfate, hydroquinone, urea, and water. Uptake of the solutions may have been too slow to permit any effect upon the rapid disease development

TABLE 12. PERCENTAGE OF WILTED FOLIAGE ON VARIOUS DATES FOR INJECTIONS BOTH BEFORE AND AFTER INOCULATION

Trees 6-10 feet tall 1942. Inoculated in the top

Treatment	Injection before inoculation							Injection after inoculation							
	Date		Days after inoculation				No. of days for 50% infection	Date		Days after inoculation					No. of days for 50% infection
	Inj.	Inoc	11	20	36	64		Inj.	Inoc	10	15	31	47	64	
Quinone	7/14	7/21	0	4.2	17	22	92	7/17	7/12	4.2	..	28	42	48	72
Hydroquinone	7/7	7/17	1.0	12	23	31.5	80	7/10	7/12	9.5	...	32.5	50	62.5	48
p Nitrophenol	7/7	7/18	.2	5	28	33	76	7/10	7/13	3.1	.	14	26.5	37.5	76
8-Hydroxyquinoline sulfate	7/7	7/18	1.7	7.8	30	39	76	7/10	7/13	5	...	14	31	40.5	88
Pyrocatechol	7/9	7/18	.3	7.1	29	45	74	7/19	7/13	5.1	...	20.6	30.5	45.5	74
Sodium thiosulfate	7/7	7/18	1.7	14	29	39	70	7/16	7/13	7.8	...	21	40.5	71	46
Pyrogallol	7/7	7/18	1.3	10	37	44	70	7/16	7/12	0.7	. .	20.4	29.4	38.8	70
Potassium permanganate	7/8	7/18	4.2	15	35	45	80	7/17	7/12	6.8	...	30	64	76	42
Vanillin	7/7	7/18	.4	10	28	39	64	7/16	7/13	3.4	...	12	41	52	64
Ascorbic acid	7/14	7/21	0	16	36	49	63	7/17	7/12	4.6	...	25	52.8	68.8	50
Benzoic acid	7/9	7/18	1.4	13	33	43	62	7/17	7/12	6.5	...	27.1	47.9	55	54
Methylene blue	7/8	7/18	1.3	17	38	47	56	7/16	7/12	4.2	...	22.5	40.1	57.5	52
8 Hydroxyquinoline benzoate	7/7	7/18	3.2	14.2	40	50	55	7/16	7/12	0	3.4	4.8	26	34	80
Hydroxylamine HCl	7/8	7/18	3.8	27.5	50	58.2	52	7/16	7/12	8.8	...	32.5	50	62	52
Quinhydrone	7/8	7/18	4	27	47	48	48	7/16	7/13	8.1	...	22	43	47	70
Phenothiazone			..	..	..	..	..	7/23	7/13	...	...	26.4	51	55	..
Check		7/17	.7	13.1	35	51	56		7/13	4.7	...	17	34	52	65

which usually follows inoculation with spores. In the other soil treatments the trees were inoculated with mycelium.

Soil applications of disodium ethylene bisdithiocarbamate (Dithane) (8) and of 8-hydroxyquinoline benzoate showed promise in 1944 (44). Thirty trees each were watered with Dithane 1-500 (two grams per liter), Dithane 1-2,000 (one gram in two liters), 8-hydroxyquinoline benzoate 1-500, 8-hydroxyquinoline benzoate 1-2,000, and water alone. One gallon per six- to twelve-foot tree was applied on June 16 and three gallons per tree on June 22 and again on July 15. The trees were inoculated in the top with a heavy spore suspension on June 21. By the end of the season (September, 1944) the number of trees showing disease symptoms in each of the treatments was as follows: Dithane 1-500, 21 or 70 per cent; 8-hydroxyquinoline benzoate 1-500, 22 or 73 per cent; Dithane 1-2,000, 26 or 87 per cent; 8-hydroxyquinoline benzoate 1-2,000, 28 or 93 per cent, and controls, 28 or 93 per cent. There was little difference in disease development on trees showing symptoms. The fact that in each case the higher concentration of the chemical caused appreciable reduction in disease occurrence, even though the trees received only seven gallons of solution, seems of significance. In this experiment the short interval between the initial application of the chemicals and the inoculation of the trees, and the low gallonage used undoubtedly did not permit optimum distribution of the chemicals through the vascular system at the vital time. Further work with either increased concentration or increased gallonage should result in more significant differences. Increasing the gallonage applied should be more satisfactory as increasing the concentration might result in injury to roots.

A fairly extensive experiment with soil application of 8-hydroxyquinoline benzoate was made in 1945 by E. M. Stoddard of this Station, who has reported the results briefly (29). He read the amount of wilting per tree as discussed in the section on methods. The data were somewhat variable because there appeared to be some difficulty in distinguishing possible wilting injury by the chemical from wilting by the disease. Nevertheless, the largest amount used per tree gave the fewest trees wilting and the least amount of wilting per infected tree.

Stoddard concluded that: "The plots treated with a single dose at all concentrations showed more trees wilting and less wilting per infected tree than plots treated with multiple doses. There was no difference between plots treated before and after inoculation either in number of trees wilting or amount of wilting per tree. From the fact that *Graphium* [*Ceratostomella*] *ulmi* was isolated from an approximately equal number of trees in all the treatments and the checks, it is suggested that the effect of the oxyquinoline benzoate was due to antidoting of the fungus toxin causing the wilting and was not due to fungicidal action".

Therapy

Approximately 500 nursery trees and a number of larger trees have been treated with various chemicals after artificial inoculation, or after noting disease symptoms in the case of natural inoculation, in attempts to cure or at least retard progress of the Dutch elm disease. The latter objective has definitely been attained, with trees ranging from four to 12 feet in height (15, 39, 41, 43, 44). Curing the disease is only a matter of retarding its progress still further. Additional work using more dosages and increased gallonage of the several effective chemicals found in this work, plus improved methods of application, should go far toward solving this problem. There is, of course, also the added possibility of finding more effective chemicals.

Results are not so definite with larger elms, although there have been some indications of favorable effect of injections for a time. However, projection of results with the small elms to larger trees indicates that it can be done. There is no vital difference between the terminal 10-foot portions of many branches on a large elm and a 10-foot seedling tree. If the 10-foot seedling can be protected, then many branches on a large tree can be protected, providing the chemical reaches the branches in the same concentration that it was present in the 10-foot seedling. This seems to be one of the prime difficulties. Several obstacles must be overcome before success can be hoped for in controlling the disease in large trees. One is that present methods of application of the chemicals do not provide adequate distribution throughout the top of the tree. The other is that by the time the disease is noted in large trees the vascular system is often heavily plugged with gums and tyloses or its function is otherwise destroyed and it is difficult to introduce appreciable quantities of chemicals. Soil applications give considerable promise of overcoming the first obstacle. Shifting the emphasis from control to prevention of the disease would overcome the second obstacle.

Most of the nursery trees used in this work were injected either at a definite interval of one week after inoculation, or were injected as wilt symptoms appeared in the individual specimens. Symptoms usually appeared within a week or 10 days following inoculation with concentrated spore suspensions.

In 1941, 130 elms from 3½ to 5 feet in height were inoculated with mycelium on bits of agar from petri dish cultures on June 18, divided into randomized groups, and injected through holes bored in the base of the stem as soon as possible after the appearance of wilt. Thirteen chemicals were compared with control trees injected with water and with uninjected controls. Wilt appeared and most of the trees were injected during the period from June 28 to July 1 inclusive. Retardation of the disease was striking in some cases (Table 13); the increase in average percentage of crown wilted from the time of injection (late June) to the end of the season (late September) was 5 per cent for trees injected with benzoic acid 1-1,000, 14 per cent for

TABLE 13. RESULTS OF INJECTING CHEMICALS INTO ELM TREES AFTER INOCULATING WITH *C. ulmi*

Chemicals were injected at the time of wilt appearance in 1941

Chemical	Concentration	No. of trees	Increase in average per cent wilt per tree from time of injection (July 1) to end of season (September 18)
Benzoic acid	1-1,000	7	5.0 per cent
Hydroquinone	1-1,500	8	14.0
8-Hydroxyquinoline benzoate	1-1,000	9	26.5
8-Hydroxyquinoline sulfate	1-1,200	9	32.8
8-Hydroxyquinoline sulfate plus urea	1-1,000 1-1,000	9	47.2
p-Aminobenzoic acid	1-50,000	9	49.0
Fumaric acid	1-1,000	8	52.0
Thymol	1-1,000	8	53.0
p-Nitrophenol	1-1,500	6	53.3
Water-injected checks	...	12	56.5
Tetramethyl thiuram-disulfide	No. 5 capsule	6	58.8
8-Hydroxyquinoline	1-1,500	8	64.4
Urea	1-500	11	64.5
Uninjected checks	...	12	68.2
Mercaptoben-zothiazole	No. 5 capsule	6	74.6

Trees 3½ to 5 feet tall, inoculated with mycelium in tops June 18, and injected in base, June 28-July 1.

trees injected with hydroquinone, 27 per cent for 8-hydroxyquinoline benzoate injections, 33 per cent for 8-hydroxyquinoline sulfate injections, 56 per cent for control trees injected with water, and 68 per cent for uninjected controls. Thymol, *p*-nitrophenol, *p*-aminobenzoic acid, fumaric acid, tetramethyl thiuramdisulfide, urea, 8-hydroxyquinoline and mercaptobenzothiazole had little effect (39).

Again in 1942 significant retardation was obtained (44). One hundred and seventy elms from six to 10 feet in height were inoculated with spore suspension in the top of the tree on July 12 and 13. The trees were then divided into groups of five, and two randomized groups of five trees were injected with each chemical. Sixteen chemicals were used; the trees were injected at the base with 500 ml. of solution July 17 to 19, before the appearance of disease symptoms. Hydroxylamine hydrochloride and methylene blue were taken up the most rapidly of all the solutions; uptake was good with 8-hydroxy-

quinoline sulfate, pyrocatechol, sodium thiosulfate, hydroquinone, and ascorbic acid. The concentration of all solutions was 1 gram per liter.

The percentage of foliage wilted per tree was read periodically during the season (Table 12) and the data were evaluated by Barratt's technique (4). Results were plotted on log.-probability paper as before and the position of the curve for each material was obtained in terms of the number of days to reach 50 per cent diseased.

It is of interest to note that the control trees inoculated July 12 required 65 days to reach 50 per cent affected, whereas control trees inoculated on July 17 (see section on Immunization) took only 56 days to reach 50 per cent. It may be significant that the trees were suffering more from lack of water on July 17 than on July 12, and hence the spores may have been more widely distributed under such conditions.

At any rate, fewer chemicals showed promise when injected after inoculation on July 12 than before inoculation on July 17. Only pyrogallol, 8-hydroxyquinoline benzoate, 8-hydroxyquinoline sulfate, and possibly *p*-nitrophenol seemed to have any curative effect.

In nursery elms from six to 12 feet in height, 8-hydroxyquinoline benzoate again showed a beneficial effect in 1944 when injected through the ends of cut branches as soon as wilt was noticed. Trees injected with the 1-1,000 dilution (average amount taken up per tree: 660 ml.) averaged 53 per cent wilt by September, those injected with the 1-5,000 dilution averaged 60 per cent wilt, while the uninjected control trees averaged 73 per cent wilt. In this experiment the trees were inoculated with approximately 200,000 spores each, in the top (1942 wood), on May 29 and injected when wilt appeared, mostly from June 7 to 9. Trees were injected with 8-hydroxyquinoline benzoate at a dilution of one gram per liter and 0.2 gram per liter, disodium ethylene bisdithiocarbamate (Dithane) at the same dilutions, "Cupferron" at one gram per liter, neutral red at 0.2 gram per liter, and α-benzyl α-phenylhydrazine hydrochloride at 0.33 gram per liter. Uptake of solutions was excellent with neutral red, trees averaging 1,155 ml.

Dithane injections resulted in nearly as much retardation of disease development as did 8-hydroxyquinoline benzoate, at the 1-1,000 dilution, with an average wilt of 55 per cent by September. Trees injected with Dithane at the 1-5,000 dilution, however, were more severely diseased than the controls. The other treatments were similar to the controls in percentage of wilt per tree.

It is evident from the curves and tables that sufficient amounts of several organic chemicals can be applied internally to small elm trees to prevent or retard progress of the vascular fungus involved, without serious injury to the host. This offers hope for future successful treatment of large trees. The most promising of the chemicals tried were: 8-hydroxyquinoline benzoate, 8-hydroxyquinoline sulfate, hy-

droquinone, *p*-nitrophenol, disodium ethylene bisdithiocarbamate (Dithane), and benzoic acid. All of these were effective either in immunization or in therapy, or both, in several cases. Some were effective more consistently than others. In seven separate injection tests with 8-hydroxyquinoline sulfate, this chemical either appreciably reduced the number of trees becoming infected or retarded disease progress markedly in five of the tests. An explanation for failure in two of the tests has already been advanced in the section on immunization (p. 54). Out of 12 tests 8-hydroxyquinoline benzoate was effective in preventing or retarding disease in eight.

If the chemicals migrate through the tree as slowly as the Dutch elm disease fungus, more work needs doing on the concentration of chemicals and timing the treatments with respect to inoculation.

Other Methods of Control

Pruning

Little or no systematic pruning of elms affected with the Dutch elm disease had been done prior to 1942. In that year experiments were begun here to determine if the disease could be controlled by pruning. Over 300 American elms, ranging in height from six to 32 feet, were inoculated with a concentrated spore suspension in the top of the main leader or in an upper branch at various times during May, June, July and August in 1942, 1943 and 1944, and were divided into groups to receive different pruning treatments. A few naturally infected trees were also pruned (44).

In 1944 May and Douglas (20) reported successful removal of infection in some cases in pruning artificially inoculated elms at the point where the inoculated branch joined the main trunk. In some cases this resulted in removing all the discolored wood; in others it did not. The disease recurred more frequently where all discoloration was not removed, as might be expected. They did not correlate effectiveness of pruning with any definite relation between location of pruning cut and discoloration in the wood.

The procedure used in experiments here was first to inoculate trees in an upper branch or leader, by putting one drop of concentrated spore suspension on the branch and cutting through it with a sharp scalpel. Spore suspensions were made up with from two to 10 million spores per ml.; each tree received approximately 1/20 ml. The trees were watched closely for development of symptoms, and generally as soon as wilt was readily evident, trees were pruned. The branches on which leaves were wilting were cut, with either pruning shears or pruning saw depending on the size of the branch, and the wood examined immediately under the bark for the brown streaks caused by *C. ulmi.* (In the case of naturally infected trees, two other vascular fungi, *Verticillium* and *Dothiorella* may cause similar

streaks.) As streaks were found, successive cuts were made down the branch until the discoloration disappeared, then a final cut was made at various test distances below this point. Test distances used were usually one, two and three feet. In one experiment only the wilted branches were removed, with no regard to removing the discoloration in the wood. Pruning tools were sterilized in 70 per cent alcohol between cuts.

In general, it was found that the Dutch elm disease can be controlled by pruning, if it is done early enough, and if trees are pruned two or three feet below the last visible (to the eye) discoloration caused by the fungus in the wood. It is important, particularly in the spring, to detect the disease in a very early stage, when a few leaves on one branch are beginning to wilt and curl. Pruning trees as soon as possible after observance of symptoms is advisable because early pruning may result in eliminating the discoloration and the fungus by removing only a small portion of the tree. As an example, nine trees inoculated on July 12, 1943, were pruned two feet below the last discoloration apparent in the wood as soon as wilt was observed. Seven other trees, inoculated at the same time, were also pruned two feet below the last signs of stain in the wood, but were not pruned until two weeks after wilt was first noticed. In the first group it was necessary to remove only 30 per cent of the tree crown to eliminate the infection; in the second group it was necessary to remove 60 per cent of the crown.

Early season infections were found to be more difficult to eradicate by pruning than those appearing in late season. In a number of cases, trees inoculated in May or June and pruned only one foot below the last visible discoloration in the wood showed disease symptoms again. There was no recurrence in trees inoculated at this season and pruned two to three feet below the last discoloration in the wood. It has been two years since some of these trees were pruned.

There are probably two reasons for recurrence of disease in trees which were pruned one foot below the last stain in the wood that could be detected by eye. The first is a gross matter of detecting discoloration. In the very early stages of development of the disease the vascular discoloration can only be seen with a microscope. Hence, a one foot margin might often not be sufficient to prevent recurrence merely because enough of the infection is not removed. The second reason, as does the first indirectly, goes back to the matter of spore dosage. In a vascular disease of this type there is movement of spores both up and down the xylem from the point of inoculation, as Banfield has shown (2). Downward distribution of spores is undoubtedly the more variable as it is apparently related to saturation deficits in various tissues. There is a possibility, of course, that spores may be distributed over much of the tree in a relatively short time. Removal of an infection by pruning may not necessarily remove all of the fungus spores from a tree. However, if only a few spores, 10 for example,

are left in the unpruned portion of the tree, the chances of these causing infection are much lower than if a larger number (1,000 for example) were left (see section on Spore Dosage). Pruning two to three feet below visible discoloration apparently reduces the spore dosage in the tree to zero or to an extremely low figure; pruning one foot below discoloration does not always result in such reduction.

July and August infections are relatively easy to remove (in the year in which they occur), because there is usually less involvement of the tree from the late season infections (see section on Time of Inoculation). There has been no recurrence of the disease in trees inoculated in July and August and pruned one, two or three feet below the last visible stain in the wood, at the appearance of wilt symptoms. Even removal of dead and dying wood at this time of year, without attempting to prune out the discoloration, has enabled 12 out of 15 trees to recover. Many trees recover naturally from a late season inoculation; pruning has resulted in a higher percentage of recoveries in our tests, however. Table 14 gives the results of several pruning experiments in 1944 with average distances of discoloration below inoculation by the time of pruning, and percentages of the tree removed. The disease reappeared in two trees which were pruned only one foot below the last discoloration in the wood.

Infections appearing in a dry season, or as the result of summer inoculations, are frequently difficult to detect. The only symptoms in these cases may be yellowing of leaves on affected branches. Even this symptom is evanescent; the leaves are soon shed, leaving merely bare branches. Similar dropping of leaves results from drouth; vascular discoloration does not occur when leaves drop from this cause, however.

The main difficulty in using this method of disease control on large elms lies in the impracticability of detecting the infection in a sufficiently early stage, so that it may be pruned out without destroying the ornamental value of the specimen. Often, by the time symptoms are apparent in a large tree, the fungus has become established throughout the greater part of the crown. As indicated above, however, even partial eradication of an infection, particularly of infections appearing in late season, may be beneficial.

Fertilization

As indicated in the section on relation of condition of the tree to development of the disease (p. 22), maintenance of a healthy condition is an important factor in resistance to development of the fungus within the tree. In the one experiment conducted here the disease did not develop as rapidly in very vigorous trees as in less vigorous trees growing in the same plot. One of the important factors contributing to vigorous growth and healthy condition is adequate fertilization; sodium nitrate and 10–10–10 fertilizers showed significant results. Further work is needed on this aspect of control, however.

TABLE 14. RESULTS OF PRUNING ARTIFICALLY INOCULATED ELMS, IN 1944

Size of trees	Date inocula-ted	Dates pruned	No. of trees inoculated	No. of trees wilting	Distance pruned below discoloration	Ave. distance discolored be-low point of inoculation	Ave. per cent of tree re-moved	Recurrence of disease in:
15-32 ft. tall	June 5	June 21 to	8	4	12 in.	31 in.	12%	1 tree
"		July 25	8	6	24 in.	25 in.	29	0
"	"	"	8	5	36 in.	20 in.	25	0
"	"	"	8	4	none—control	...	...	...
8-12 ft. tall	June 16 (in '43	June 29 to	10	10	12 in.	15 in.	29%	1 tree
"	wood)	July 18	10	10	24 in.	10 in.	32	0
"	"	"	10	8	36 in.	9 in.	48	0
"	"	"	10	9	none—control	...	...	...
8-12 ft. tall	June 16 (in '42	June 29 to	5	5	12 in.	13 in.	36%	0
"	wood)	July 18	5	5	24 in.	19 in.	64	0
"	"	"	5	5	36 in.	11 in.	61	0
"	"	"	5	5	none—control	...	...	...
8-12 ft. tall	June 30	July 10 to	10	2	12 in.	11 in.	25%	0
"	"	July 23	10	3	24 in.	13 in.	40	0
"	"	"	10	1	36 in.	18 in.	50	0
"	"	"	10	4	none—control	...	...	...
"	July 18	...	40	0	...	...	...	...

Development of Resistant Elms

Observations of inoculations in the nursery block of elms at Mt. Carmel and of other trees in experimental plots, and of the development of natural infections in Connecticut, indicate that there is considerable variation in the progress of disease in individual seedling American elms (*U. americana* L.) Numerous data have been taken showing similar sized trees wilting appreciably less than others which received the same dosage of spores, particularly in late season inoculations. Extreme variations are not common, but differences occur frequently enough with seedlings to make it desirable to use at least 10 and preferably more trees in comparative experiments.

Variations are undoubtedly present in development of the disease in naturally infected elms, but it is usually impossible to determine in these cases whether the inoculation conditions were comparable, as they were in the inoculations of nursery elms. A tree dying back severely from the disease may have received a very heavy load of spores in a number of inoculation courts, or may have been more

susceptible at the time of inoculation because of defoliation by insect pests, or both. Another naturally infected tree may be lightly diseased because it received only a few spores from a few beetles, or because it was maintained in an exceptionally vigorous condition.

The reasons for the existing differences between individual American elms in resistance to the disease may be morphological or chemical, or both. There is an indication of more rapid development of the disease in trees with a wide band of spring vessels than in those with a relatively narrow band. A chemical difference is also very likely.

Smucker (26) has shown that several exotic species and varieties of elm are resistant to the disease. These include the *Ulmus pumila* group and *Ulmus foliaces* var. Christine Buisman. It is possible that a satisfactory resistant tree may be found or eventually developed in a breeding program. This approach may well solve the Dutch elm disease problem in the future, but unfortunately has little application to the thousands of valuable *Ulmus americana* specimens already planted.

SUMMARY

The Dutch elm disease is a serious problem in Connecticut where elms are so prominent in the landscaping scheme, as well as in many other sections of the eastern United States. Coming on the heels of the chestnut blight, the discovery of the disease called forth many dire predictions in the early 'thirties on the fate of the elms.

In retrospect, it is clear that the disease has not been as totally destructive as feared, but has necessitated development of measures aimed at learning how to "live with the disease". Research was expanded here in 1940 concomitant with the decline in the eradication program. The objectives were to investigate the growth of the disease in the elm population of the State, to investigate the biology of the fungus, the pathology of the sick plants, and the possibilities of disease control by vascular chemotherapy.

Satisfactory nutrient solutions were developed for growing the fungus. The most satisfactory solid medium, for both growth and spore production, is malt extract agar. The zonate character of the growth of the fungus on agar plates was found to be a response to alternate light and dark periods.

Introduction of suspensions of spores of the causal fungus (*Ceratostomella ulmi*) directly into xylem vessels, severed with a sharp scalpel or knife, was found to be the most effective method of inoculation. The involvement of the tree was increasingly rapid and effective as the location of the inoculation moved down from the top toward the base of the tree.

Inoculations made in the period of active spring growth (generally mid-May to late June) were the most effective, both in regard to percentage of trees showing symptoms and to development of the

disease in the tree. Summer inoculations were usually rather ineffective and commonly did not carry over to the next season. In years of high summer precipitation, inoculations in summer were of more consequence, a fact that may be correlated with the type of growth of the host.

The probability of infection was found to be proportional (probably logarithmically) to the number of spores used in inoculation. Trees receiving light dosages of spores showed either no external symptoms or light symptoms, and usually outgrew such attacks the following year. Increasing the number of inoculation points intensified the effectiveness of any given spore dose.

Indications were obtained that effectiveness of spores in producing disease decreases as the spores increase in age.

The fungus was found to produce a toxic substance or substances in culture which is evidently the main factor in producing disease symptoms in the tree. Symptoms were reproduced by injecting sterile filtrates from cultures of the fungus into elms. Production of the toxin was favored by using asparagine as a nitrogen source.

The condition of the tree is very important with respect to development of the disease. Elms which had been severely defoliated by spring cankerworms were much more seriously affected when subsequently inoculated with the fungus than were non-defoliated trees. Trees fertilized with sodium nitrate and 10–10–10 fertilizers were less severely diseased than unfertilized checks or trees fertilized with urea or ammonium sulfate. Vigor of growth was an important factor; the most vigorous trees, regardless of type of fertilization, were much less affected by the disease than those in poorer vigor.

An extensive study was made of the quantitative aspects of disease advance: (1) within the sick tree, (2) outward from a sick tree, and (3) through a town or the State.

Studies on the rate of involvement of the tree showed a logarithmic dimensional equivalence of distance, time, spore load and resistance of the vessels to transmission of water. The results do not quite agree with what would be expected from hydraulic theory which postulates arithmetic dimensional equivalence of time, distance and pressure. Presumably, the hiatus that introduces the logarithmic factor is an exponential rate of vascular plugging due to the formation of tyloses and gums.

The spread outward from a tree varies inversely as the logarithm of the distance, which, of course, is another statement of the inverse square law. The practical result is that, in the areas studied, the chances that a tree would become infected at 50 feet were about 3.5 in 10, at 100 feet, one in 10, and at 500 feet, one in 500.

Intensification of the disease in a township (or town) was arith-

metic with time. Whether the growth of the disease in a town is in accordance with a logistic or probability function could not be determined with the data available.

The Dutch elm disease has spread through Connecticut arithmetically with time and at an approximately constant rate of 5.4 miles per year along radii of a circle centered at Battery Park in New York City, which is at the assumed port of entry of the disease into the country.

Considerable effort has been made to evaluate with data now available the giant roguing experiment conducted during the 'thirties to control Dutch elm disease. Few, if any, felt in 1933 and 1934 that the roguing experiment should not be started. However, the evidence now available suggests that nature overrode the best efforts that could be devised. The slopes of the curves of the advance of the disease within a town or across the State do not become steeper with 1940 when the roguing experiment stopped. Published data from other areas are insufficient for this type of mathematical treatment.

The heterocyclic 8-hydroxyquinoline group and the triphenyl methane dyes were the most effective of over 100 organic chemicals tested against *C. ulmi in vitro.* Several chemicals in these two groups inhibited growth at dilutions of 1 to 50,000 or more. Several organic chemicals were found to neutralize the fungus toxin in culture.

The possibility of preventing or controlling the disease by introducing chemicals into the vascular system of elm trees was investigated extensively. Approximately 50 chemicals, largely organic, were used in various tests from 1940 to 1944. Phytotoxicity was relatively low, particularly with the organic chemicals.

The majority of the chemotherapy work was done with nursery elms (*Ulmus americana*), 4-12 feet in height, growing under closely comparable conditions. Approximately 1,100 of these trees were treated in various ways either before or after inoculation with *C. ulmi.* A number of larger trees were also treated. Methods included direct injection of trunk, injection of cut ends of branches, soil applications, and spraying. Soil applications appear promising from several aspects.

It was found that sufficient amounts of several organic chemicals can be applied internally to small elm trees to prevent or retard progress of the vascular fungus involved, without serious injury to the host. Similar results should be possible with large trees providing several obstacles are overcome, such as inadequate distribution of chemicals through the top of large trees and extensive plugging of the vascular system. Soil applications give promise of overcoming the first obstacle; shifting the emphasis from control to prevention of disease would overcome the second.

The most effective of the chemicals tested were 8-hydroxyquin-

oline benzoate, 8-hydroxyquinoline sulfate, hydroquinone, *p*-nitrophenol, benzoic acid, and Dithane (disodium ethylene bisdithiocarbamate). In research on prevention of the disease, a protective effect was obtained with these chemicals when injected into trees or when watered in basins at the base of the trees before inoculating with the fungus. With smaller seedlings, the "immunization" was striking in some cases. Soil applications of 8-hydroxyquinoline benzoate and Dithane showed promise on six to 12 foot trees in 1944. In therapy experiments, similar definite trends in retarding disease progress were obtained with the above chemicals and with pyrogallol.

The progress of this vascular disease in a tree during a normal growing season was found to bear a linear relation to the logarithm of time, when the percentage of infection per tree was plotted as probits against the logarithm of the number of days since inoculation took place. This provides a graphic means of studying effectiveness of chemical treatments in retarding disease progress, by comparing curves for disease progress over the season in control trees versus treated trees. From these curves the number of days to reach a given percentage of disease was obtained; the best treatments would obviously be those delaying development of disease for the longest time.

A number of the treatments produced disease-progress curves of flatter slope than the controls, and markedly increased the interval between inoculation and 50 per cent infection. Included in these were injections with 8-hydroxyquinoline sulfate and benzoate, hydroquinone, *p*-nitrophenol, pyrogallol, and quinone; most of these were used at a concentration of one gram per liter. Both slope and number of days to reach a given disease level (50 or 95 per cent) should be considered in judging efficiency of the chemical.

It was found that the Dutch elm disease can be controlled by pruning, without eradicating the entire tree, if the pruning is done at a very early stage. Early detection of disease and treatment is particularly important with infections taking place in the spring. Pruning two to three feet below the last visible discoloration in the wood was uniformly successful in preventing recurrence of the infection.

Considerable variation was noted in the response of seedling American elms to artificial inoculation with *C. ulmi*, indicating the possibility of ultimately developing an American elm resistant to the disease.

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